FORSCHUNGEN
AUF DEM
GEBIET DER PFLANZENKRANKHEITEN

Herausgegeben von

Prof. Dr. Shigeyasu Akai

Universität zu Kyoto

Heft V, Nr. 4

Kyoto, Japan

1956

Inhaltsübersicht

目　次

Antibiotic production by *Gloeosporium olivarum* cultured on media containing 2,4-D*

Nakato NAITO and Toshikazu TANI

With 7 tables and 4 figures

1. Introduction

As to the mechanism of the activity of the plant growth-regulators, including 2,4-D, to higher plants, there have been published up to date a large number of papers discussed from various points of view, although as yet a clear conclusion has not been obtained. Whereas many investigations relating their effect on fungi or bacteria have also been made especially in recent years, almost nothing is known of the process in them. The authors (7, 13) have pointed out previously that the inhibitory activity of 2,4-D on the growth of *Gloeosporium olivarum* had progressively increased as the culture period prolonged. In view of such finding there arose the question that the inhibition by 2,4-D may not depend on its immediate influence against the causal fungus but may be due to a certain substance capable of inhibiting its own growth induced by the addition of 2,4-D, the suspected substance being increased with the progress of culture. Several recent investigations undertaken along this line (9, 10, 11) have clearly demonstrated that an antibiotic considered to be responsible for the inhibition by 2,4-D is isolated from the culture filtrates of the pathogen which was grown in the presence of 2,4-D. These research findings were published in Japanese. Since the fungistatic or fungicidal activity of chemicals commonly employed for the control of crop diseases is generally accepted to be attributable to their direct action against the pathogen, this specific mechanism of the inhibition by 2,4-D as well as the fact that the causal fungus is easily induced to form an antibiotic on media supplied with 2,4-D in spite of the nonproduction on those without 2,4-D, should excite much interest. The present paper gives an outline of the results of the writers' experimental studies concerning this problem.

2. Purification of antibiotic produced by *Gloeosporium olivarum* on media containing 2,4-D

In a preliminary study (10) it was confirmed that a certain fungistatic substance is present in the culture filtrate of *Gloeosporium olivarum*, the causal fungus of the olive anthracnose, grown on media containing 2,4-D, being nondetectable from the harvested mycelia. Therefore the fractionation of filtrates with the different solvents was performed in order and we succeeded in the isolation of an active crystalline product. The causal fungus was maintained in quiet culture for 24 days at 25°C in 100 Erlenmeyer flasks of 200 cc capacity, each containing 50 cc of the liquid media supplied with 0.04 % sodium 2,4-dichlorophenoxyacetate (2,4-D). Unless otherwise indicated, the standard culture

* Contributions from the Laboratory of Phytopathology, Kagawa Agricultural College, Prefecture Kagawa, Japan.

Received July 20, 1955

medium mentioned in this paper is as follows: peptone 20g, KH_2PO_4 1g, $MgSO_4 \cdot 7H_2O$ 0.4g, 2% $FeCl_3$ aqueous solution 3 cc, sucrose 50 g, dist. water 1 L. The initial pH of the medium was 5.4. About 4.4 L of the filtrate thus obtained has been treated following the scheme presented in Fig. 1, each fraction being analyzed for its inhibitory effectiveness by means of the dilution agar media technique or paper disk method.

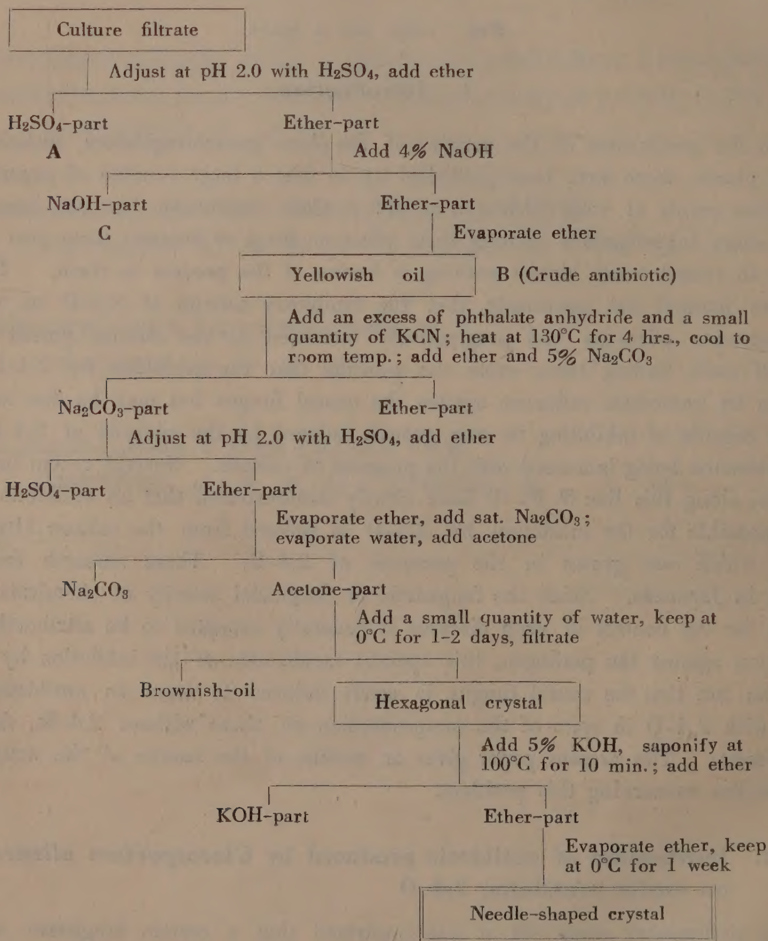


Fig. 1. Schematic summary of the purification process of the antibiotic.

When ether was added to the filtrate acidified to pH 2 0 with H_2SO_4, the water soluble fraction (Fig. 1, A) in which the residue of nutrients transfered did not develop inhibition at all. From the ether soluble fraction shaken with 4% NaOH aqueous solution, about 1.1 g of the crude antibiotic (B) was obtained as a yellowish oil after evaporation of ether. Marked inhibition resulted from this material. Although NaOH soluble fraction (C) also slightly retarded the growth of the fungus, the resulting effect would be probably due to 2,4-D since it has been ascertained that 2,4-D moves to this fraction. On these grounds above stated, the crude antibiotic of a yellowish oil was considered to play a main role in the inhibitory activity of the culture filtrates. Since the crude material in question reduced

Nessler's solution and gave positive alkalixanthate reaction, indicating that material of the hydroxyl group characteristic to a primary or secondary alcohol is present, it was converted into phthalate derivative and then induced in the crystalline product as its Na salt, the details being described in the following lines.

Thus, adding an excess of anhydrous phthalic acid and a small quantity of KCN to the crude substance, the mixture was heated in a glycerine bath for 4 hrs., then shaken with 5% Na_2CO_3 and ether. After ether was again added to the Na_2CO_3 soluble phase acidified to pH 2.0 with H_2SO_4, the ether solution was washed with water and dried over anhydrous Na_2SO_4 to an orange-yellow oil; Na_2CO_3 and next acetone were added to it after evaporation of water, then filtered with filter paper. If the resulting filtrate was supplied with a small amount of water after evaporation of acetone, and allowed to stand, crystallization set in. Filtering with 1-G-4 glass filter yielded approximately 180 mg of hexagonal plate crystals (Fig. 2), m. p. 58°–58.5°C. After recrystallization by addition of a small quantity of water, it was saponified with the supply of 5% KOH in a boiling-water bath for 10 min. and shaken with ether. When the ether solutions were further washed with water, dried over anhydrous Na_2SO_4 and kept for about 7 days in a refrigerator at about 0°C, the antibiotic in question was finally isolated as colourless needle-shaped crystals (Fig. 3), m. p. about 34°C. The yield was approximately 75 mg.

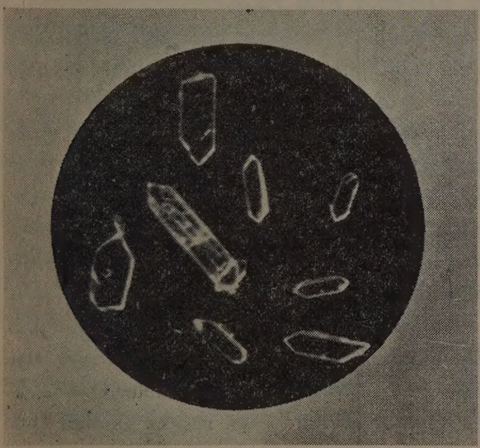


Fig. 2 Crystal of sodium salt of phthalic ester of the antibiotic.

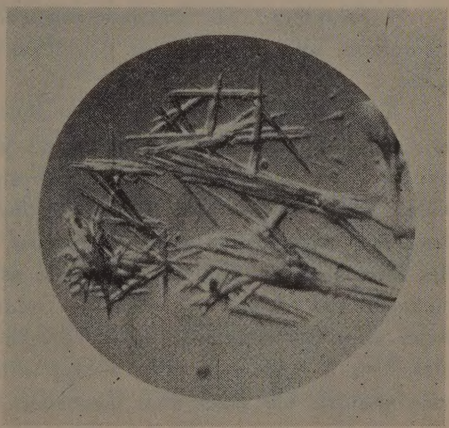


Fig. 3 Crystal of the purified antibiotic.

Rf of this pure antibiotic estimated by paper chromatography was identical with that of the active factor in the crude product as will be seen later. Hence it is fairly clear that the active agent existing in the crude material has remained unaltered during the subsequent purification steps. There occurred here a reasonable question whether the antibiotic now obtained might be induced by impurities because the experiments hitherto described had been performed with the commercial material of 2,4–D which contains about 88.86% sodium 2,4–dichlorophenoxyacetate. Since, however, crystallized sodium 2,4–dichlorophenoxyacetate purified from the commercial material was also proved to be similarly effective in inciting the inhibition as well as the antibiotic production, it is beyond doubt that the production of the antibiotic concerned is not due to impurities. In addition, it is also apparent that the production of the antibiotic is not merely a single reaction between

2,4-D and nutrients initially added, because none was formed even if liquid media supplied with 2,4-D were kept for 30 days at 25°C without inoculation of the pathogen. At the same time another factor that the causal fungus might essentially have a nature to evolve a staling substance injurious to its own growth was easily neglected since such substance was not detected at all in the control culture without the supply of 2,4-D. In view of these facts now stated, the antibiotic would presumably be an abnormal metabolic by-product of the fungus owing to 2,4-D.

3. Some chemical and physical properties of the antibiotic

1. Stability. Stability tests were carried out by paper disk method with the antibiotic preparations at various temperatures and pH levels. The results showed that the inhibitory activity for the causal fungus was not destroyed by exposure to 105°C for 30 days or 140°C for 4 hrs. It was also found to be stable in the wide range of hydrogen-ion concentrations, since no inactivation occured at least at pH 4.0–11.2 tested.

2. Quantitative test. The purified antibiotic reduces Nessler's reagent and is positive for alkali xanthate and xanthoprotein reaction. It is easily soluble in conc. H_2SO_4, resulting in a red colouration. The ninhidrin, diazotized benzidine, Millon and Liebermann (for nitroso compounds) test are negative. It gives no colour with $FeCl_3$.

3. Solubility. The antibiotic concerned is easily soluble in ether, acetone, ethanol and chloroform, soluble in benzene, petroleum ether and hot water and almost insoluble in cold water.

4. Paper chromatography. One-dimensional paper chromatography studies were made as means of comparing the crude and pure antibiotics. Ascending chromatograms were run for 7 hrs. at about 20°C on "Tōyōroshi" No. 50 filter paper strips 40 cm long and 1 cm wide. Strips were spotted with the testing material which were carried in dilute methanol, dried and suspended in a sealed chamber, the atmosphere of which had been saturated with the developing mixture. The mixture used contained 30 per cent acetone, 5 per cent methanol and 65 per cent water, by volume. The movement of the active factor on the paper strips was determined by the portion of the inhibition zone when the filter paper was placed on a large assay plate seeded with conidia of *G. olivarum*. The determination of zone positions was made after incubation for 48 hrs. at 25°C. The results showed that the inhibition zone presented in strips of the crude material was only one and that R_f of the spot was quite identical with that of the pure substance, both being 0.78. Hence the pure antibiotic is recognized to play a principal role in the virulence of the crude material.

5. Taste. The pure substance has a bitter taste on touching the tongue. The similar taste of the crude material might be due to this pure antibiotic.

4. Fungistatic and bacteriostatic activity of the antibiotic

1. Effect upon the growth. In order to ascertain the toxicity of this purified antibiotic against the causal fungus and *Pythium aphanidermatum*, it was incorporated into agar media in various dilutions ranging from 1:5,000 to 1:50,000 and the growth on them was compared in 50 cc flasks, estimating the diameter of colonies after incubation at 25°C for 3 days in the former fungus and 36 hrs. in the latter one. On account of its insolubility in cold

water, the substance concerned was added to flasks as an acetone solution and acetone was evaporated before media were poured into them. The mean value of 3 flasks per each dilution is summarized in Table 1. The figures in the table show a numerical value obtained by subtracting the size of inocula from the diameter of colonies measured. It is here seen that both fungi are unable to grow at 1 : 5,000, the limiting dilution for the presence of the inhibitory effect probably being about 1 : 50,000. Since 2,4-D permits somewhat the growth of the causal fungus even at the higher level of 0.32% as previously reported (7), the fungistatic potency of the antibiotic can be said to be far more severe than that of 2,4-D.

Table 1. Diameter in mm of colonies of *Gloeosporium olivarum* and *Pythium aphanidermatum* on a peptone-salts agar medium containing the antibiotic of different dilutions.

Fungus	Antibiotic dilutions							Control without antibiotic
	1:5,000	1:7,500	1:10,000	1:20,000	1:30,000	1:40,000	1:50,000	
G. olivarum	0.0	7.0	9.1	15.7	16.8	17.3	18.7	21.5
P. aphanidermatum	0.0	5.5	12.8	28.3	33.0	39.5	45.8	47.5

Another examination regarding the effect on microorganisms was also performed by paper disk method. The test organisms included 7 species of plant pathogenic fungi and 4 bacteria, 2 of which are plant pathogens. In the case of fungi, square filter paper of 1 cm² containing respectively 0.3, 0.1, 0.03 and 0.01 mg of this substance was placed on agar plate at the distance of 3 mm from the margin of colonies which had been previously grown until the size of about half of a Petri dish. After being incubated then at 25°C for 5 hrs. in *Pythium aphanidermatum*, 36 hrs. in *Piricularia oryzae* and 24 hrs. in the other fungi, the width of a clear zone (the distance of the foremost edge of the paper and the edge of the mycelial mat) was estimated. In bacteria, on the other hand, their suspension was poured on the Bouillon's agar and allowed to dry with the removal of the supernatant. Soon after the circular filter paper of 1 cm² respectively containing 0.6, 0.3, 0.1 and 0.03 mg was then laid on that agar plate, the diameter of the inhibition zone was measured incubating for 24 hrs. at 30°C.

It is obvious from the data in Table 2 that the antibiotic is effective against all fungi examined at the level exceeding 0.3 mg and against bacteria above 0.1 mg, with only one exception of *Phytomonas destructans* in which the growth was entirely unaffected.

Table 2. The width or diameter in mm of the clear zone formed between the paper disc containing the antibiotic and the colonies of the microorganisms.*

Microorganism	Micrograms of the antibiotic per disc				
	0.6	0.3	0.1	0.03	0.01
Gloeosporium olivarum		3.0	1.5	±	–
Corticium centrifugum		2.5	0.5	–	–
Macrosporium porri		2.5	±	–	–
Cochliobolus miyabeanus		2.5	0.5	–	–
Piricularia oryzae		2.0	1.5	–	–
Sclerotium hydrophilum		2.5	0.5	–	–
Pythium aphanidermatum		2.5	1.5	–	–
Erwinia aroideae	16	16	–	–	
Phytomonas destructans	–	–	–	–	
Escherichia coli	20	19	±	–	
Erwinia subtilis	18	14	–	–	

* The figures in fungi present the width of the clear zone and those in bacteria present the diameter of that.

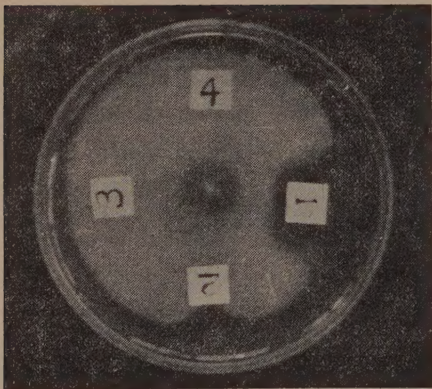


Fig. 4 Effect of the paper disc containing the purified antibiotic of different amount upon the growth of *Pythium aphanidermatum*.
1. 0.3 mg 2. 0.1 mg 3. 0.03 mg
4. 0.01 mg

A similar experiment carried out with the crude product indicated that its potency in the paper disk method is always somewhat lower than that of the corresponding level of the pure product whereas the potency of both preparations is almost the same in the dilution method. This difference according to the testing method may be explained as that the impurities of the crude material affected the diffusion rate of its active factor in the case of paper disk method. On the basis of the agreement of the potency in the dilution method for both preparations and the results obtained by the paperchromatography showing that the pure antibiotic is the principal factor for the virulence of the crude antibiotic, it is presumed that the impurities are not comprised so much in the crude material.

2. Effect upon the conidial germination. The effect of the antibiotic upon the germination of conidia was studied with *G. olivarum* and *Cochliobolus miyabeanus*. The conidial suspension of both fungi was dropped on a thin layer of the agar media which contained the pure substance in various dilutions. After being allowed to dry with the removal of the supernatants, it was kept under saturated humidity at 25°C. Table 3 shows germination readings at 12 hrs. in the former fungus and at 10 hrs. in the latter one. Thus, conidia of *G. olivarum* were unable to germinate at 1 : 5,000 and 1 : 7,500, the germination being only 2.0% even at 1 : 10,000. The germ-tubes attained the mean length of 11.1μ, but failed to develop further even at 48 hrs. *C. miyabeanus* also almost did not germinate at 1 : 5,000, the germination being 14.7% at 1 : 10,000. The germ tubes in comparison with control are very short in this fungus too.

Table 3. Germinaton of conidia of *Gloeosporium olivarum* and *Cochliobolus miyabeanus* on a peptone-salts agar medium containing the antibiotic of different dilutions.

Fungus	Dilution of the antibiotic	Number counted	Number germinated	Per centage germination	Length of germ-tubes (μ)
G. olivarum	1 : 5,000	476	0	0.0	—
	1 : 7,500	520	0	0.0	—
	1 : 10,000	617	12	2.0	11.1
	Control	390	382	98.0	76.6
C. miyabeanus	1 : 5,000	573	1	0.2	28.5
	1 : 7,500	265	4	1.5	28.5
	1 : 10,000	347	51	14.7	81.3
	Control	344	318	92.4	121.9

5. Effect of 2,4-D concentration and culture period on the antibiotic production

The relative titer of the antibiotic produced under the different concentrations of 2,4-D as well as culture period was compared by bioassay. The lst culture was carried out at

room temperature on 50 cc of liquid media having an initial pH of 5.4 in 200 cc Erlenmeyer flasks which contained 0.04, 0.06 and 0.08% 2,4-D respectively. Withdrawing 5 flasks per each concentration after incubation of 12, 16, 20 and 24 days, the crude antibiotic (Fig. 1, B) was extracted from its own filtrates, incorporating it in new agar media of which the quantity is identical with the original filtrates. The 2nd culture of the causal fungus was conducted at 25°-30°C on these media. The relative potency of the antibiotic produced in the 1st culture was measured by index calculated by dividing the diameter of colonies on the 2nd culture after incubation of 7 days by that of control media without 2,4-D, multiplying the quotient by 100. The dry weight of the mycelium, pH value and reaction to Fehling's solution of the filtrate were also determined at the end of the 1st culture for the sake of the experimental criticism. The general relationships are obvious from Table 4.

Table 4. The antibiotic production in relation to 2,4-D concentration as well as culture period.

Concentration of 2,4-D added (%)	The 1st culture				The 2nd culture	
	Age of culture (day)	Dry weight of mycelia per flask (mg)	pH of filtrate after culture	Fehling reaction of filtrate	Diameter of colonies (mm)	Growth index against control
0.0	0				54.4	100.0
	12	1,604.5	6.4	−	51.5	94.7
	16	1,484.3	7.8	−	51.3	94.3
	20	1,355.6	7.0	−	52.0	95.6
	24	1,340.2	7.1	−	53.0	97.4
0.08	12	31.8	4.4	+	22.7	41.7
	16	35.3	4.4	+	15.3	28.1
	20	37.1	4.4	+	6.1	11.2
	24	36.6	4.4	+	5.1	9.4
0.06	12	61.4	4.4	+	17.1	31.4
	16	53.1	4.4	+	13.7	25.2
	20	70.0	4.4	+	8.0	14.7
	24	45.6	4.4	+	6.2	11.4
0.04	12	119.6	4.4	+	11.5	21.1
	16	100.1	4.3	+	13.6	25.0
	20	121.9	4.4	+		
	24	88.5	4.3	+	3.3	6.1

The results of the 1st culture indicate that in control media autolysis has already begun on the 12th day, while in those 2,4-D added not only much of the sugar yet remains unused but also the pH value is maintained almost constant until the end of the culture for 24 days, although slightly declined to acidic side. Namely on media with 2,4-D the growth ceases in course of culture notwithstanding the onset of autolysis owing to the exhaustion of nutrients seems unlikely. This suggests that the titer of the antibiotic at the incubation period of 12 days is enough to cause the entire suppression of the growth. The relative titer of the antibiotic rose steadily at least until the 24th day in any concentration of 2,4-D. Moreover the titer at 0.04% was presumed to be higher than 0.06% and 0.08% in any growth period. In another experiment which was studied in the concentrations of 0.04, 0.02 and 0.01%, the peak seemed to lie at 0.04%. From the results of these two experiments, the optimum concentration of 2,4-D for the antibiotic production seems to be 0.04%. On the contrary, the growth of the causal fungus on media supplid with 2,4-D has been proved to decrease progressively with the increase of

concentration as previously reported (7, 13). This fact, therefore, seemingly appears to be inconsistent with the authors' opinion later described that the inhibitory activity of 2,4–D is mainly due to this antibiotic instead of its direct action against the pathogen, since the yield of the antibiotic also should progressively increase as the concentration of 2,4–D increases providing the authors' opinion is true. Presumably it could be explained by the assumption that the absolute amount of the antibiotic in concentrations higher than 0.04% is small in consequence of the unfavourable growth due to its rapid production.

6. Effect of nutrients on the antibiotic production

Experiments were made to obtain information on the relation between nutrients and the antibiotic production.

1. The kind of the medium. The relation between the kind of media and the occurence of the antibiotic was studied by the bioassay method mentioned in the previous section, using 6 kinds of liquid media. Media supplied with 2,4–D to give 0.04% were adjusted to pH 5.4 with HCl or NaOH and on 50 cc of them in 200 cc flasks the 1st culture of the pathogen was carried out for 12 days at 25°C. The crude antibiotic extracted from the filtrates of 4 flasks per each medium was added to agar media, the 2nd culture of the causal fungus being conducted for 7 days at 25°C on them. As shown in Table 5, soy media and synthetic media with peptone gave highest yield of the antibiotic.

Table 5. The antibiotic production on several kinds of liquid media.

Kind of medium	The 1st culture		The 2nd culture	
	Dry weight of mycelia per flask (mg)	pH of filtrate after culture	Diameter of colonies (mm)	Growth index against control
Potato decoction	680.8	5.8	42.0	89.2
Soy	84.6	4.6	5.9	12.5
Czapek	435.0	7.0	48.4	102.8
Asparagine-salts	513.6	5.4	28.4	60.3
Richards	518.5	7.0	46.1	97.9
Peptone-salts	152.3	4.2	7.4	15.7
Control			47.1	100.0

2. The nitrogen source. The relation of nitrogen source to the production of the antibiotic was studied by growing the pathogen in Richards' solution which contains one of several nitrogenous compounds instead of KNO_3. To give the same level of N with that of KNO_3, they were added to media as follows: $NaNO_3$ 8.4 g, $CO(NH_2)_2$ 3.2 g, NH_4NO_3 4.0 g, ammonium tartarate 10.6 g, asparagine 7.4 g, $(NH_4)_2SO_4$ 6.6 g per 1 L of solution. The antibiotic titer of the 1st culture was estimated by bioassay on the 2nd culture quite similar to the previous experiment. The results obtained are in Table 6. These data show that the most productive source of nitrogen was $C_4H_4O_6(NH_4)_2$, but good production was obtained with NH_4NO_3, $(NH_4)_2SO_4$ and asparagine; but other sources gave poor results.

3. The carbon source. The relation of carbon source was also examined by the same method with the foregoing experiments excepting that the 1st culture was carried out on a modified Richards' solution in which glucose, fructose, starch, mannit or glycerine (25 g/L) was used as the sole carbon source. As shown in Table 7, the best were sucrose and glucose but starch and fructose were good; glycerine and mannit very little or none. In addition it was also evident from Nessler's reaction that autolysis had not yet set in.

Table 6. The antibiotic production on media containing different sources of nitrogen.

Nitrogen source	The 1st culture: Dry weight of mycelium per flask (mg)	The 1st culture: pH of filtrate after culture	The 2nd culture: Diameter of colonies (mm)	The 2nd culture: Growth index against control
$NaNO_3$	781.8	7.2	45.9	97.5
$CO(NH_2)_2$	415.8	6.0	39.7	84.3
NH_4NO_3	17.3	4.4	4.3	9.1
$C_4H_4O_6(NH_4)_2$	77.1	4.6	1.5	3.2
Asparagine	115.6	4.6	9.8	20.8
$(NH_4)_2SO_4$	19.8	4.2	7.4	15.7
Control			47.1	100.0

Table 7. The antibiotic production on media containing different sources of carbon.

Carbon source	The 1st culture: Dry weight of mycelia per flask (mg)	The 1st culture: pH of filtrate after culture	The 2nd culture: Diameter of colonies (mm)	The 2nd culture: Growth index against control
Glucose	114.6	4.2	14.4	25.4
Fructose	108.8	4.6	28.6	50.4
Sucrose	54.5	4.2	12.4	21.9
Starch	216.4	4.7	23.7	41.8
Mannit	326.0	6.6	56.9	100.4
Glycerine	444.6	5.2	48.0	84.7
Control			56.7	100.0

The above 3 experiments relating the effect of nutrients on the antibiotic appearance are almost consistent in the respect that the titer was higher when the pH value was maintained in the lower level during the culture. The authors (8) have previously pointed out that 2,4–D exhibits an increasing inhibition as the pH value during culture decreases, and that the cause might be attributable to the lower dissociation of 2,4–D in acidic side, only their undissociated molecules being absorbed into mycelia. The facts above stated would probably show that the yield of the antibiotic is influenced by the amount of the 2,4–D molecules absorbed into mycelia according to the pH value during culture.

7. Mechanism of the inhibitory activity of 2,4–D against *Gloeosporium olivarum* and its significance

Literature has accumulated, particularly in recent years, dealing with the effect of the plant growth-regulators on microorganisms but nothing is known of the mechanism of their inhibitory activity. So far as the writers know, the report of Anker (1) that the respiration of *Saccharomyces cerevisiae* was stimulated by heteroauxin is the single one concerning this problem. It is described in the present paper that an antibiotic is purely isolated from the culture filtrates of *G. olivarum* which was grown on media supplied with 2,4–D. Not only are any inhibitory substances almost undetectable in the culture filtrates except for the crude antibiotic concerned on the one hand, but also the antibiotic purely isolated is presumed as the main agent responsible for the activity of the crude substance in view of the results of paper chromatography on the other hand. Consequently the authors consider that the inhibitory activity of the culture filtrates is mainly attributable to this purified antibiotic.

Furthermore, the present as well as previous studies concerning the mechanism of the

inhibitory activity of 2,4–D to *G. olivarum* provide five lines of evidence which led the authors to conclude that 2,4–D has no inhibiting action of its own on the growth of the pathogen directly but reveals its effect by producing this antibiotic.

(1) The growth of the causal fungus, in general, is retarded on media containing 2,4–D owing to this antibiotic produced in the culture filtrates. According to the cultural condition, however, the unfavourable growth does not occur even if 2,4–D is added on media and at the same time in such culture filtrates fungitoxic substances are almost not produced.

(2) The inhibitory activity of 2,4–D increases with the progress of culture. This is in agreement with the increasing yield of the antibiotic as the culture advances.

(3) The fact that the inhibition by 2,4–D is significant on media of which pH value was maintained at lower level, is consistent with the result that this antibiotic is elaborated only on media of which pH value was similarly low during culture.

(4) When two colonies of the present fungus are cultured together on agar media without 2,4–D in the same Petri dish, they come into direct contact. However on media 2,4–D added, they are separated by an inhibition zone with the width of 1.0–3.0 mm even at the end of culture for 30 days, probably in consequence of the production of this antibiotic.

(5) Although 2,4–D of comparatively lower concentrations does not show an inhibition at the incubation of 7 days, suggesting that the inhibition by 2,4–D does not depend on its direct influence upon the pathogen, the growth of fungi is forced to be suppressed probably owing to the accumulation of this antibiotic if the subsequent culture is continued.

When a filter paper of 1 cm^2 containing 2,4–D of 0.1 mg was placed on agar plate at the distance of 3 mm from the edge of mycelial mat which had been previously grown up to about half of a Petri dish, the mycelia extended on the upper and lower sides of paper on the next day, showing no inhibition. On the contrary, in the case of paper similarly supplied with a trace of the antibiotic, an inhibition zone was clearly observed already on the following day. When the paper supplied with 10 mg of 2,4–D was laid on agar plate and then H_2SO_4 was poured on it after 24 hrs., it could be easily seen with the naked eye that crystallized 2,4–D was diffused around the paper disk in the circle of 3 cm in diameter. The concentration of 2,4–D in the diffused area at 1 mg presents 0.035% when calculated on the basis of the diffusion area at 10 mg. The concentration in the practical case is assumed to be higher than 0.035%, since the degree of diffusion would reasonably be greater as the amount added is larger. However, even provided that the diffusion in both cases is quite identical, this concentration of 0.035% proved to be enough for the prevalence of inhibition at least after the incubation of 5 days, if 2,4–D of that level was added to media. These observations furnish additional evidence that 2,4–D may not reveal the direct inhibition against the pathogen.

Chemicals commonly employed for the control of crop diseases have been generally considered to exert their inhibitory action by affecting the pathogen itself. Hence this specific mechanism of the inhibitory activity presented by 2,4–D must be said to be very interesting. Moreover the authors' attention is particularly attracted to the fact that 2,4–D induces the causal fungus to form an antibiotic in the presence of 2,4–D in spite of non-appearance on the normal media. It is probable that the mechanism of inhibition for other fungi, the growth of which is proved to be retarded by 2,4–D, is similar with that

for the present fungus, if not identical.

As to the mechanism of the activity of the plant growth-regulators to higher plants, there have been published a large number of papers discussing the subject from various points of view, although as yet a clear conclusion has not been obtained. Several investigators, however, put forward an opinion that the herbicidal activity of 2,4–D is due to the accumulation of a phytotoxic substance incited by its treatment. For example, FULTS et al. (3) demonstrated this view with respect to scopoletin and van OVERBEEK et al. (17) also supported this idea. Such assumption also agrees with the report by AUDUS et al. (2) who stated that the toxic action of 2,4–D resembles that of coumarin and also with the investigation by HAMNER et al. (5) that β–methyl umbelliferone, a coumarine derivative, is more toxic to Gramineae than to broad-leaved plants. Since it is also known, on the other hand, that in plants generally a small guantity of coumarin derivatives is detected (4, 15) and that they are toxic to plants as well as phytopathogenic fungi (5, 6, 14, 16) this assumption regarding the mechanism of the 2,4–D activity to higher plants would call for special notice. Although it is not certain now whether the antibiotic in question is a coumarin derivative or not, the above assumption must be said to be consistent with the authors' opinion in the respect that the cause was ascribed to a product incited by abnormal metabolism of organisms.

There are many reports that the treatment of plants by 2,4–D decreases or increases plant diseases. The senior author et al. (12) have also previously reported that 2,4–D spray on rice plants decreases lesions by *Cochliobolus miyabeanus*. However still more research will be needed to get a correlation between this mechanism of 2,4–D action and the decrease of plant disease.

Résumé

An antibiotic substance was purely isolated as colourless needle-shaped crystals from the culture filtrate of *Gloeosporium olivarum* Alm. causing the olive anthracnose which was grown in the presence of 2,4–D. The purified antibiotic is soluble in organic solvents and insoluble in cold water; thermostable and not inactivated at the pH range between 4.0 and 11.2. M.P. is about 34°C.

When the antibiotic was incorporated into the synthetic agar media with peptone, it completely inhibited the growth of *Gloeosporium olivarum* and *Pythium aphanidermatum* at the dilution of 1 : 5,000. The experiment by paper disk method indicated that the substance are similarly inhibitory against the growth of other 4 species of phytopathogenic fungi and 3 bacteria. Conidia germination of *G. olivarum* and *Cochliobolus miyabeanus* was also entirely or almost inhibited at 1 : 7,500.

The relative titer of the antibiotic rose steadily with the progress of culture at least until the 24th day in any concentration of 2,4–D added. The optimal concentration of 2,4–D for the antibiotic production was 0.04% and the yield was also maximum in a modified Richards' solution. As to the mechanism of the inhibitory activity of 2,4–D to *G. olivarum*, there have been provided five lines of evidence that 2,4–D exhibits an inhibition on the pathogen by this antibiotic produced in its presence rather than a direct action of 2,4–D.

References cited

1. ANKER, L.: Proc. K. Nederland Akad. Wetenshap Amsterdam 52(8): 875–881, 1949.
2. AUDUS, L. and QUASTEL, J. H.: Nature 159: 320-324, 1947.
3. FULTS, J. and JOHNSON, M.: West. Weed Conf. Denver. January, 1950.
4. GOODWIN, R. H. and TRAVES, C.: Amer. Jour. Bot. 37: 224–231, 1950.
5. HAMNER, C. L., SELL, H. M., KLOMPARENS, W. M. and VAUGHN, J. R.: Bot. Gaz. 112 (2): 135–137, 1950.
6. ISHII, Y.: Jour. Agri. Chem. Soc. Japan 27(11): 777–780, 1953, (in Japanese).
7. NAITO, N. and TANI, T.: Tech. Bull. Kagawa Agr. Coll. 3(2): 80–90, 1951, (in Japanese).
8. ——, ——: Ibid. 5 (2): 164–174, 1953, (in Japanese).
9. ——, ——: Ibid. 7 (1): 41–50, 1955, (in Japanese).
10. ——, ——: Ann. Phytopath. Soc. Japan 19 (3,4): 129–132, 1955, (in Japanese).
11. ——, ——: Jubilee Publication in Commemoration of the Sixtieth Birthdays of Prof. Yoshihiko TOCHINAI and Prof. Teikichi FUKUSHI. Japan: 185–189, 1955, (in Japanese).
12. —— and TAKAI, S.: Tech. Bull. Kagawa Agr. Coll. 6 (1): 66–79, 1954, (in Japanese).
13. —— and KUNIKATA, H.: Forsch. Gebiet Pflanzenkr., Kyoto, 5 (3): 113–118, 1955, (in Japanese).
14. SAN ANTONIO, J. P.: Bot. Gaz. 114(1): 79–95, 1952.
15. STOLL, A., PERIERA, A. and RENZ, J.: Helvetica Chim. Acta 33(6): 1637–1647, 1950. (Biol. Abst. 25 (3), no. 9207, 1951).
16. THIMANN, K. V. and BONNER, W. D.: Proc. nat. Acad. Sci. U. S. A. 35, 272–276, 1949.
17. OVERBEEK, J. v., BLONDEAU, R. and HORNE, V.: Plant Physiol. 26(4): 687–696, 1951.

Studies on the Nature of Disease Resistance in Plants
A contribution to the knowledge concerning the mechanism of infection of plants by the weak pathogenic fungi under snow-cover and the nature of disease resistance against fungi or viruses on the basis of the results of histo- and cytochemical observations.

Tokuzo HIRAI *

The first series of this investigation were designed to obtain information on the nature of resistance of wheat plants to the diseases caused by the weak pathogenic fungi, *Typhula incarnata* and others. The diseases, so-called snow-blight diseases of winter cereals, the pathogens of which have been identified as *T. incarnata*, *T. ishikariensis*, *Sclerotinia graminearum*, *Fusarium* spp., and *Pythium* spp., widely distribute throughout the north-eastern regions in Japan. The causal fungi can only infect the plants which weakened under snow-cover, and not infect the healthy plants. Question that might be asked is that why a healthy plant is not infected by the fungi. It is from this view-point that consideration was given in the following study to determine the weakened conditions of host plants under which the pathogens can infect, and the nature of pathogenicity of the weak pathogenic fungi.

1. The weakened conditions of host plants under snow-cover

An approach to this problem is to determine:

(1) The seasonal change and varietal difference in contents of sugars and nitrogen compounds of wheat plants under snow-cover (6). In each of these experiments samples were taken from the plants under snow-cover at the times indicated, and the above-ground parts of the 5 to 15 plants were ground up as finely as possible with stirring rod in a mortar and extracted for analysis, in a green state. Usually 5 to 6 varieties, differing as to resistance to snow-damage, were used. Reducing and non-reducing sugars were determined by Bertrand's method, proteinous and non-proteinous nitrogen compounds by Kjeldahl's semi-micro method, and amide and ammonia nitrogen compounds by Magnesium oxide method.

The amount of sugar concentrations decreased gradually during the course of snow-cover in all varieties tested; although a rapid decrease could be observed after two months since snow-cover. In addition, the snow-resistant varieties always contained much higher amount of them than the less resistant (Table 1).

The content of both protein and non-protein nitrogen compounds showed hardly change in the initial stage of snow-cover; whereas the stage of a decreasing in the former content was found to be in accordance with that of a rapid decreasing in the sugar content. The non-protein nitrogen concentrations giving rise in the same stage were also observed probably owing to the decomposition of protein. Therefore, the data reveal that the snow-resistant

---

* Laboratory of Plant Pathology, Faculty of Agriculture, Nagoya University, Anzyo, Japan.
Received Aug. 3, 1955.

Table 1. Seasonal change in amount of sugar concentrations in wheat varieties under snow-cover.

Sugars	Varieties	Date of sample selection*				
		25, Nov.	25, Dec.	25, Jan.	25, Feb.	25, Mar.
Non-reducing	snow-resistant	2.14[a]	1.84	1.40	0.34	0.15
	less-resistant	1.12[b]	1.03	0.92	0.29	0.03
Reducing	snow-resistant	1.48	1.34	1.08	0.40	0.15
	less-resistant	1.15	0.88	0.59	0.38	0.03

* 25, Nov.—before snow-cover; 25, Dec. to 25, Mar.—under snow-cover.

a. Percentage per green weight. Figures represent the average value of the three resistant varieties.

b. Ditto. Figures represent the average value of the three less-resistant varieties.

varieties have the higher ratio of both protein and non-protein nitrogen to the green weight than the less resistant during snow-cover (Table 2).

Table 2. Seasonal change in amount of proteinous and non-proteinous nitrogen compound concentrations in wheat varieties under snow-cover.

Nitrogen compounds	Varieties	Date of sample selection*				
		25, Nov.	25, Dec.	25, Jan.	25, Feb.	25, Mar.
Proteinous	snow-resistant	0.67[a]	0.67	0.68	0.60	0.51
		72.8[b]	74.4	75.6	71.4	65.4
	less-resistant	0.62	0.63	0.63	0.56	0.42
		80.5	78.8	79.7	75.7	66.7
Non-proteinous	snow-resistant	0.25	0.23	0.22	0.24	0.27
		27.2	25.6	24.4	28.6	34.6
	less-resistant	0.15	0.17	0.16	0.18	0.21
		19.5	21.2	20.3	24.3	33.3

* See the footnote in table 1.

a. Percentage per green weight. Figures represent the average value of three varieties.

b. Percentage per total nitrogen.

The contents of ammonia and amide nitrogen compounds changed very irregularly; nevertheless it seems to have tendencies of increasing in the amount of the former compounds especially in the less-resistant varieties under the last stage of snow-cover (Table 3).

Table 3. Seasonal change in amount of ammonia and amide nitrogen compound concentrations in wheat varieties under snow-cover.

Nitrogen compounds	Varieties	Date of sample selection*				
		25, Nov.	25, Dec.	25, Jan.	25, Feb.	25, Mar.
Ammonia	snow-resistant	0.07[a]	0.07	0.07	0.17	0.12
	less-resistant	0.10[b]	0.04	0.13	0.13	0.25
Amide	snow-resistant	0.06	0.09	0.16	0.08	0.25
	less-resistant	0.12	0.05	0.08	0.15	0.16

* See the footnote in table 1.

a. Permillion per green weight. Figures represent the average value of the three resistant varieties.

b. Ditto. Figures represent the average value of the three less-resistant varieties.

In conclusion, these results seem to make it possible to confirm the facts that the contents of sugars and non-protein nitrogen compounds of the snow-resistant varieties are higher compared with the less-resistant even before the snow-fall; consequently, the amount of synthesized protein is also higher in the former varieties, being in continuation of these conditions throughout the periods (Figure 1).

(2) Changes in the osmotic pressure and protoplasmic permeability in wheat cells under snow-cover (16). Osmotic

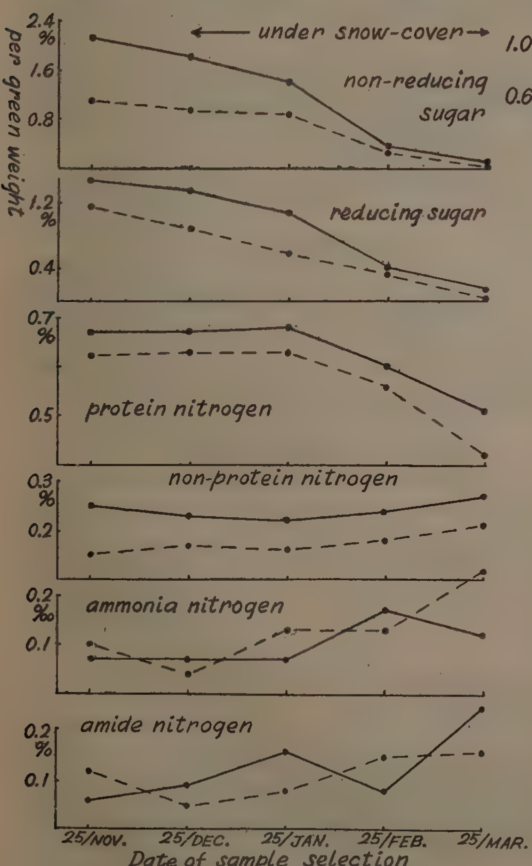


Fig. 1. Seasonal change and varietal difference in contents of sugars and nitrogen compounds of wheat plants under snow-cover.
——•—— Varieties resistant to snow-damage
-----•----- Less resistant varieties

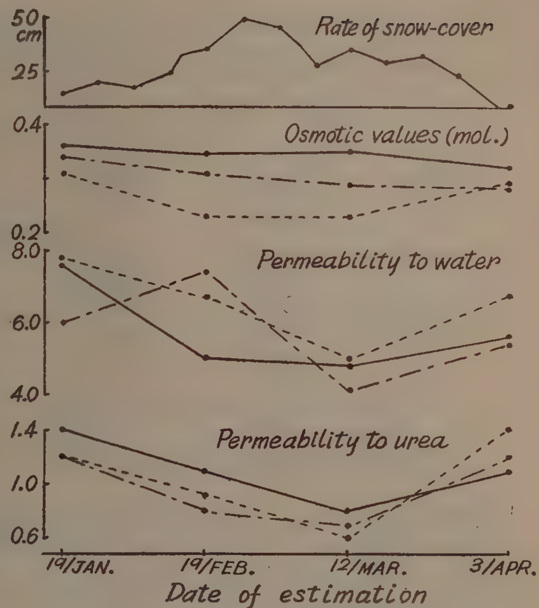


Fig. 2. Changes in the osmotic pressure and the protoplasmic permeability in wheat cells under snow-cover.
——•—— Norin No. 24, resistant to snow-damage
—·—•—·— Shinichi, comparatively resistant
-----•----- Nitta-wase, less resistant

pressure of the epidermal cells within a leaf-sheath of wheat plants under snow-cover was periodically estimated by plasmolytic method, and the cell permeabilities (both to water and urea) were also estimated by the end-plasmolytic method (20).

Osmotic value was always higher in the resistant varieties than in the less-resistant, while its seasonal change could not be confirmed. Both the water and urea permeabilities, on the other hand, were tend to decrease gradually under snow-cover, then increasing rapidly after snow-melt. Concerning the relations between the varietal difference of cell permeabilities of wheat plants, no closer correlations were found (Table 4).

Table 4. Changes in the osmotic pressure and the protoplasmic permeabilities in the wheat cells under snow-cover.

	Varieties*	Date of estimating **			
		19, Jan.	19, Feb.	12, Mar.	3, Apr.
Osmotic values	Norin No. 24 Shinichi Nittawase	0.36 mol 0.34 0.31	0.34 0.31 0.23	0.35 0.29 0.23	0.32 0.28 0.29
Permeability to water	Norin No. 24 Shinichi Nittawase	0.076 0.060 0.075	0.050 0.074 0.067	0.048 0.041 0.050	0.056 0.054 0.067
Permeability to urea	Norin No. 24 Shinichi Nittawase	0.014 0.012 0.012	0.011 0.008 0.009	0.008 0.007 0.006	0.011 0.012 0.014

* Norin No. 24, resistant to snow-damage; Shinichi, comparatively resistant; Nittawase, less-resistant.

** Date of beggining of snow-cover: 10, Dec. and date of snow-melt: 29, Mar.

The results of the experiments are illustrated in figure 2. These findings led to conduct further experiments to test the physical states of protoplasm which weakened under snow-cover and as its results would become susceptible to the fungi.

(3) Increased viscosity of protoplasm in a starved cell and the general considerations regarding the nature of physiological weakening of wheat plants under snow-cover (5). To certain whether the physical states of protoplasm do change in a starved conditions, it appeared desirable to compare those of the healthy and starved cells with regard to the displacement by centrifuging, rounding-up time on plasmolysis, and the deplasmolysis injury.

When a main-stem of wheat plants was cut and starved at 20°C for three days, the displacement of protoplasm in the epidermal cells was found to be less by the same centrifuging force than of the healthy (not starved) protoplasm (Table 5).

Table 5. Displacement by centrifuging of protoplasm in the epidermal cells of detached leaf-sheath of wheats.

Varieties	Treatment	Nos. of cells observed	Nos. of cells with displaced protoplasm	Percentage of displacement
Norin No. 24	starved healthy	373 432	226 392	60.1 % 90.7
Norin No. 58	starved healthy	543 517	350 465	64.4 89.9
Nishi-mura	starved healthy	515 564	241 438	46.7 87.6
Nitta-wase	starved healthy	300 285	207 270	69.0 94.5

Table 6 shows the same results when the wheat plants grown in soil were interrupted from sunlight by a black box.

Table 6. Displacement by centrifuging of protoplasm in the epidermal cells of leaf-sheath of wheats grown under dark.

Dark period	Plants	Treatment	Nos. of cells observed	Nos. of cells with displaced protoplasm	Percentage of displacement
days					
25	barley	dark	716	373	52.1 %
		control	787	705	89.6
	rye	dark	752	472	62.8
		control	749	686	91.6
60	barley	dark	720	414	57.5
		control	720	670	93.1
	rye	dark	719	328	45.6
		control	720	632	87.8

Using 0.6 to 0.8 M $CaCl_2$ or sucrose as plasmolyte, more rapid rounding up of the healthy than of the starved cells was observed. The healthy cells tend to plasmolyse convexly, while the starved cells mostly concavely. Using sucrose, it was also found that the starved cells were 44 to 70 per cent killed on deplasmolysis from a 1.0 M solution, and 60 to 70 per cent killed from 2.0 M. The healthy cells, on the contrary, showed only 7 to 26, and 10 to 46 per cent killing respectively. From these data, the author has arrived at the conclusion that the viscosity of protoplasm may be increasing in the starved conditions.

A second point which deserves emphasis is that the linked series of cellular changes which may occur associating with the weakening of plants have become increasingly clear; incidental to this, the author should like to present the following scheme as the process of a physiological degeneration of plants under snow-cover:

Breakdown of carbohydrates ⟶ Dissociation of protein molecules ⟶ Increased viscosity of protoplasm ⟶ Decreased permeability of protoplasm ⟶ Weakening of plants ⟶ Increased susceptibility against fungi.

The previous report (17) concerning the weakening of plants under snow-cover mostly involved the hypothesis in which the resultant production of ammonia by the broken down of protein and its damegeous injury to cells are two major factors. The hypothesis have seemingly not been considered as a suitable basis for experimental works. As far as the present evidence points out, the author could not fully in agreement with them.

2. The nature of pathogenicity of a weak pathogenic fungi, *Typhula incarnata*

In past, some attention had been paid to the toxic substance secreted by phytopathogenic bacteria or fungi. In case of snow-blight fungi, the author, in the same way, found that the culture filtrate of the fungi causes a lethal effect on wheat cells when they were grown on a Waksman's solution. Some of the data on the cultural conditions favoring the secretion of a toxin and properties of a substance derived from it will be now presented.

(1) Factors influencing the toxin production by a snow-blight fungus (14).

The results of preliminary tests have indicated that a toxic substance is usually produced when the fungus was grown in plant decoction, such as wheat and barley, and not

produced when it was in the synthetic medium except a Waksman's solution. The pH of the culture filtrate changes to acidic three weeks after incubation, producing a toxic substance at the same time. This substance was heat stable and produced abundantly when used peptone as nitrogen source and glucose as carbon source. Tables 7–9 are showing the results of experiments on the relations between toxin production of the causal fungus and nitrogen or carbon sources in medium.

Table 7. Relation between the growth and toxin production of the snow-blight fungus and nitrogen source in medium.

Nitrogen source *	Final pH of medium **	Toxin production after 4 weeks	Dry weight of mycelium in mg
NH_4Cl	5.6	±	6.9
$(NH_4)_2SO_4$	5.6	±	5.1
$(NH_4)_2CO_3$	5.8	±	8.5
$C_2O_4(NH_4)_2$	6.0	±	4.4
KNO_3	5.6	−	1.0
$NaNO_3$	6.0	−	3.7
$Ca(NO_3)_2$	5.4	−	6.5
NH_4NO_3	5.4	−	4.1
$(NH_2)_2CO$	6.4	−	4.4
Glutamic acid	5.4	−	7.4
Asparagine	5.4	+	1.9

* Used as nitrogen source instead of peptone in Waksman's solution.

** Initial pH was adjusted to 6.2–6.3.

Table 8. Relation between the growth and toxin production of the snow-blight fungus and sugars in medium.

Sugars *	Final pH of medium **	Toxin production after 4 weeks	Dry weight of mycelium in mg
Glycerine	6.4	−	63.3
Mannit	6.4	−	78.6
Arabinose	6.4	±	42.3
Xylose	6.3	−	18.2
Glucose	5.0	+	118.7
Fructose	5.8	±	98.2
Galactose	6.4	±	40.1
Mannose	5.0	−	89.9
Sucrose	5.2	±	87.4
Lactose	6.4	−	32.7
Maltose	6.4	−	67.6
Soluble starch	6.4	−	—
Celullose	6.4	±	—
Inuline	6.4	±	40.5
Dextrine	5.8	±	66.0

* Used instead of glucose in Waksman's solution.

** Initial pH was adjusted to 6.2–6.3.

Although vitamin B_1 was able to be a growth-promoting factor to the fungus, toxin production was not influenced by its addition in medium. There was no correlation between the autolysis of the fungus and the toxin production, indicating that it must be secreted as exotoxins.

(2) Properties of the substance involved (15). The toxic substance could be dialysable through a cellophane membrane, showing it is a modrately small molecular weight. It was not adsorped by carbon powder and appeared to be soluble in alcohol and ether, but not in acetone. No precipitation by lead acetate or basic lead acetate was observed.

Table 9. Relation between the growth and toxin production of the snow-blight fungus and organic acids in medium.

Organic acids *	Final pH of medium **	Toxin production after 4 weeks	Dry weight of mycelium in mg
Formic acid	6.4	±	27.7
Lactic acid	6.4	−	28.5
Oxalic acid	6.4	±	18.1
Succinic acid	6.6	−	33.4
Fumaric acid	6.4	±	18.8
Malic acid	6.4	±	12.6
Tartalic acid	6.4	−	23.8
Citric acid	7.0	±	24.5

* Used instead of glucose in Waksman's solution.

** Initial pH was adjusted to 6.2–6.3.

With the aid of these information, the author made a attempt to isolate the causal substance and obtained it as crude which was sparingly soluble in water, showed acidic upon dissolution, and was still injurious to wheat cells when diluted 1 to 10 (Figure 3).

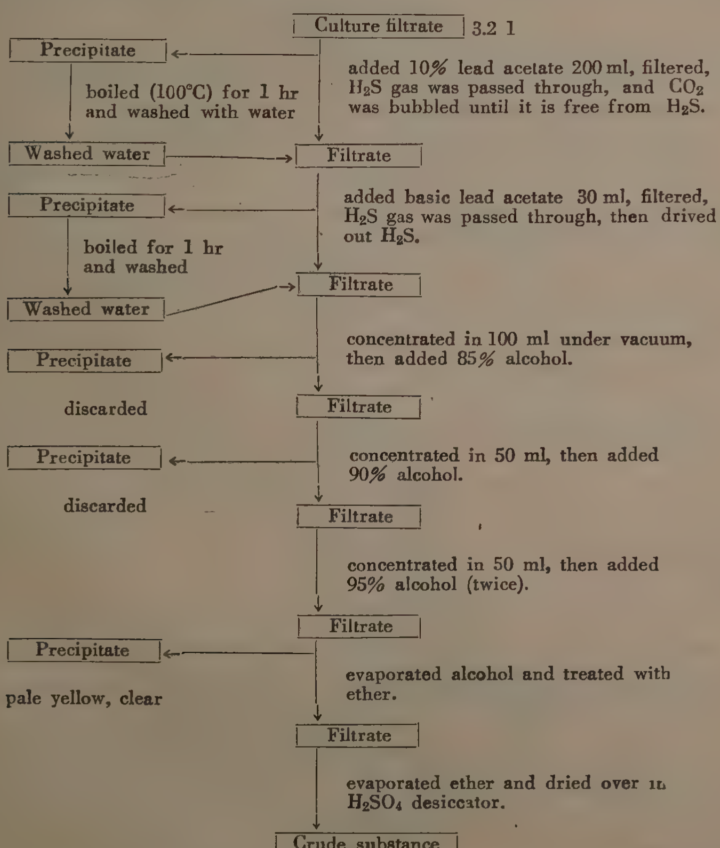


Fig. 3. Isolation of a toxic substance from the cultural filtrate of the snow-blight fungus.

It is of especial interest that these resultant substance, which now considered to be a toxic product of metabolites produced by the weak pathogenic fungus, may be a simple acidic substance; regardless of the many facts that high polymers such as protein, peptide, or polysaccharides, were definitely responsible for a toxic substance derived from a more strongly pathogenic fungus (13, 18).

In continuation of his previous studies, the author's discussion will be devoted, in the second series of this investigation, to the nature of disease resistance on the basis of the results of histo- and cytochemical observations. In such instances, it appeared desirable to examine the resistance of virus diseases as well as that of fungal diseases, always comparing them each other. The following experiments have been undertaken to test this problem and for its possible solution.

3. Necrotic reactions as a factor for resistance in plants

(1) Histochemical study upon the browning tissues of wheat plants which induced by the invasion of snow-blight fungi (9). A main-stem of wheat plants grown under snow-cover was cut off from its base and the leaf-sheath was carefully removed. The inner part of the sheath was inoculated with a agar block of fungus culture and placed in moist chamber in an incubator. After a suitable period, the epidermal layer was stripped with a razor and examined microscopically after treated with various reagents (19).

In the resistant varieties, most striking of all was that the epidermal cells into which the infection hyphae penetrate, turn to reddish brown soon after the fungal invasion. The marginal cells adjacent to the necrosis were proved to be alive in those varieties, but not in less resistant. This was confirmed from the observations on the protoplasmic streaming and using phase microscopy (Tables 10–11). Necrotic tissues and especially healthy cells surrounding them have showed positive to various reactions, such as Millon, Ehrlich's indole, ninhydrin, Molish, phloroglucin-HCl, orcin-HCl, fuchsin-SO_3, Sudan, Serra, and Nadi; while the cells invaded by fungal hyphae without necrosis did not (Table 12).

Table 10. Protoplasmic streaming in barley cells next to necrotic regions.

Cells	Rate of streaming (μ/sec.)	
	range	average
Healthy	4.5 — 5.0	4.8
Next to necrosis (1)	4.1 — 5.2	4.6
Do. (2)	4.1 — 6.0	5.0

Table 11. Protoplasmic streaming in non-invaded barley cells next to the infection (without necrosis).

Cells	Rate of streaming (μ/sec.)	
	range	average
Healthy (control) (1)	6.1 — 7.4	6.8
Do. (2)	5.6 — 6.4	5.7
Do. (3)	5.3 — 5.5	5.4
Next to infection (1)	3.7 — 4.9	4.4
Do. (2)	1.8 — 5.8	3.5
Do. (3)	3.4 — 5.6	4.6
Do. (4)	2.5 — 4.6	3.7

Table 12. Histochemical reactions upon the tissues around the necrosis.

Reactions	Substances reacted	Rate of reactions in the tissue	
		non-invaded (around the necrosis)	invaded (without necrosis)
Millon	tyrosine	+	−
Ehrlich's indole	indole	+	±
Indophenol	〃	+	
Diazo	phenol	±	−
$FeCl_3$	〃	±	−
Ninhydrin	amino acids	+	−
Molish	sugars	+	−
Seliwanoff	〃	+	±
Phloroglucin-HCl	pentose	+	±
Orcin-HCl	〃	+	−
Fuchsin-SO_3	aldehyde ?	+	−
Sudan	lipid	+	−
Nadi	oxidase; quinone ?	+	−

Previous investigator (4) has reported that in highly resistant plants, the parasite kills cells as soon as it attempts to penetrate them. The death of some of the cells would change metabolic process in the surrounding cells so that their vacuolar sap become very rich in phenolic compounds, making those cells most uncogenial for the pathogen.

As far as the author's materials concern, the Diazo and $FeCl_3$ tests which show the presence of phenolic compounds were less conspicuous and the fungi did not instantly kill cells. Opposed to that, notably in the case of resistant varieties, the healthy cells around the infection, in where the granular fraction (probably mitochondria or microsome, and not vacuole) would, as one of their components, actually participate, reacted hypersensitively before their death, and turn to reddish brown being dissociated to amino acids, lipids, pentose, and phosphorus. It might be well to emphasize the fact that the substances showing the various reactions described above seem to be originated from and closely allied to protein and nucleic acid which are the essential constituents for mitochondria or microsome in cells.

(2) Histochemical study of local lesions on potato plants incited by potato viruses (8). It has been well known that some of the potato varieties, when inoculated with juice containing potato virus X or Y, show the local necrotic lesions on the leaves. An adequate examination on some histochemical reactions of the lesions will contribute materially to our understanding of this phenomenon.

The Millon, xanthoprotein, ninhydrin, diazo, and nitro reactions were confirmed both on the necrotic regions and the healthy cells adjacent to them. Nadi and peroxidase

Table 13. Histochemical reactions upon the tissues around the necrosis caused by virus.

Reactions	Substances reacted	Rate of reactions in the tissues		
		necrotic	healthy next to necrosis	healthy
Millon	phenol		+	−
Xanthoprotein	protein	+		−
Ninhydrin	amino acids	+		−
Eisencyan	reductive subs.		+	−
Diazo	phenol	+	+	−
Ehrlich	indole		−	−
Nitro	〃	+		−
Nadi	?		+	−
Peroxidase	oxidase	−	+	+

reactions, on the contrary, proved to be negative upon the necrosis, and this facts are well accord with the staining observations using oxidation-reduction dyes that the necrotic tissues are reductive, and their adjacent cells oxidative (Table 13).

From the standpoint of histochemical reactions appearing on the affected tissues, the reliable comparisons between the defense mechanism of host plants against viruses and fungi may be successfully taken. An extensive study has made clear that the formation of local lesions in viral diseases has its developing process inseparable from that shown by the brown necrosis in fungal diseases in many respects. In both instances, the surrounding tissues just beyond the necrosis presented the same reaction providing the evidence of presence of protein, nucleic acid, and their derivatives. Furthermore, emphasis must be laid upon the dynamic processes in which these substances were being oxidized, through the sequence of successive reactions, to quinone and the related compounds [21].

4. Hypersensitive cell reactions as a factor for resistance which will be formed before the appearance of brown necrosis

Many observations that brown necrosis did not always represent the host resistance, have recently been conducted [10]. In some case, mycelial growth was confirmed penetrating through a brownish tissues; and in others, fungi seem to be destroyed by host tissue not exhibiting any detectable necrosis. From these reason, it may give general support to the conclusion that browning reaction did not necessarily determine host resistance. We must sought, therefore, precisely a pre-browning response which will appear in cells soon after a hyphal invasion.

(1) Cytochemical observations upon the hypersensitive reaction caused by the invasion of snow-blight fungus [7]. When a hypha enters a cell, it was observed that protoplasmic granules, presumably mitochondria or microsome themselves, do react and enclose the infection sites in case of highly resistant varieties. These sites take up well basic dyes after a Garnoy's fixation, and this cytoplasmic basophilia was removed by ribonuclease (RNA-ase) pretreatment (Table 14). On the other hand, the acidic dyes, such as acid

Table 14. Staining of nucleic acid in the tissues around the infection.

Substances stained	Not treated, but stained with			Pretreated before staining with	
	Thionin	Pyronine-methyl green	Azur I	TCA-acid*	RNA-ase **
Cell membrane	violet	violet	violet	+	+
Granules accompanied with hyphae	greenish blue	pink	greenish blue	+	+
Infection site	〃	violet	〃	+	+
Host nucleus	blue	blue	blue	+(−)	+(−)
〃 nucleolus		pink		−	
Hyphae	violet	〃	violet	−	−
Inner necrosis	blue	〃	blue	−	−
Cytoplasm near the infection	〃	〃	〃	−	−

* 5% trichloroacetic acid, at 90°C for 15 min.

** 0.1% ribonuclease, pH 6.0, at 60°C for 3 hrs.

fuchsin and so on, stain them red, as control sections, even after RNA-ase pretreatment. The results just outlined indicate that both ribonucleic acid and basic protein would exist

in sites of the plasm granules. Likewise, oxidative aud alkali phosphatase reactions were found to be positive on the same loci, indicating a shifts in oxidation relationship and an increase in some of the enzyme activities upon these sites (Table 15).

Table 15. Alkali phosphatase reaction in the tissues near the infection.*

Substances stained	With substrate	No substrate	Pretreated with citrate buffer	Heated	Calcium test
Nucleus					
in the healthy	−∼±	−	−	−	−
near infection	+	±∼−	+	−	−
Cytoplasm near the infection	+	±∼−	+	−	±∼−
Granules accompanied with hyphae	+	+	−	+	+
Infection site	+	+∼±	−	±	+
Cell membrane	+	+	−	+	+

* Substrate, β-glycero phosphate (sodium), glycine buffer of pH 9.4.
Citrate buffer, pH 4.5 for 15 min.
Heated, at 90°C for 2 min.
Calcium test, treated with saturated lead acetate and ammonium sulfide.

(2) Cytochemical observations upon the host-virus interactions, with special reference to the formation of inclusion bodies caused by various viruses (11). In the present section, chemical nature of some inclusion bodies found in the cytoplasm of leaf epidermal cells will be investigated. The samples mostly selected for this study were wheat plants infected with Triticum Virus 1A and tobacco plants with Solanum Virus 1.

Sections pretreated with trichloroacetic acid (TCA), perchloric acid (PCA), hydrochloric acid, or ribonuclease (RNA-ase) in order to remove nucleic acid were stained with trypan blue. The X-bodies were still stained as were those in the control sections indicating that the dye was staining the protein. The TCA- or PCA-pretreated nuclei and X-bodies were not stained with pyronine-methyl green; while the HCl- or RNA-ase pretreated nuclei stained light-blue with the same dye, and the bodies treated the same remained unstained (Table 16-17). It would thus seem to indicate from these results the presence of ribonucleic acid in the bodies.

Table 16. Staining of nucleic acid in the X-bodies.

Treatments	Staining with		
	Trypan blue	Giemsa	Pyronine-methyl green
Not treated			
nuclei	light blue	blue	green
bodies	violet	pink	pink
crystal*	blue	pink	pink
HCl-treated			
nuclei	light blue	blue	light blue
bodies	violet	pink	none
crystal	blue	pink	none
TCA-treated			
nuclei	light blue	light pink	none
bodies	violet	pink	none
crystal	blue	pink	none
PCA-treated			
nuclei	light violet	light pink	none
bodies	violet	pink	none
crystal	violet	pink	none

* means crystalline inclusions found in cytoplasm.

Table 17. Ribonuclease pretreatment before staining of nucleic acid in the X-bodies.*

Treatments	Staining with				
	Thionin	Azur I	Methylene blue	Unna	Acid fuchsin
Not treated					
nuclei	violet	blue	blue	violet	light purple
bodies	green	green	green	pink	purple
cell membrane	light violet	blue	blue	violet	none
RNA-ase pretreated					
nuclei	light violet	light violet	blue	blue	light purple
bodies	none	none	none	none	purple
cell membrane	light violet	blue	blue	purple	none

* Fixed with Carnoy's fluid ; RNA-ase treatment, 0.5 %, pH 7.0, at 60°C for 2 hrs.

The Millon reaction, biuret test, xanthoprotein test, azo reaction, ninhydrin test, and Thomas' test for arginine have all proved to provide evidence of protein in the bodies. The X-bodies caused by wheat virus were stained with Sudan III reaction proving the presence of lipids. They were not stained in Feulgen reaction. The crystal inclusions possessing the properties of protein were also found in the cytoplasm or within the nuclei. (Table 18).

Table 18. Cytochemical reactions upon the X-bodies caused by Triticum Virus 1A.

Reactions	Substances reacted	Results	Color
Millon	tyrosine	+	red
Biuret	peptide	+	violet
Xanthoprotein	tyrosine	+	orange
Azo	tyrosine, histidine	+	pink
Ninhydrin	amino acids	+	violet
Thomas	arginine	+	red
Rhomieu	tryptophane	−	yellow
Nitro	indole	+	yellow
Ehrlich	〃	−	〃
Liebermann	〃	−	〃
Phloroglucin-HCl	pentose	−	none
Orcin-HCl	〃	−	〃
Sudan III	lipids	+	red
Osmic acid	〃	+	brown
Phospholipids	〃		
extracted by acetone		+	blue
not extracted		+	purple
Serra	organic phosphorus	−	yellowish
Feulgen	DNA	−	none
Nadi	oxidase	+	violet
Dopa-oxidase	〃	+	brown
Alkali P-ase	phosphatase	+	black
Acid P-ase	〃	+	brown

It is quite probable from the results of experiments that the inclusion bodies caused by viruses may be the host production formed as a result of host-virus interactions. Because that some evidence on the existence of viruses within the bodies has now become conclusive (3), the possible explanation that these bodies might be the production of host hypersensitive reaction, whose constituent derived from host cells will be a mitochondria or microsome fraction, would make some contribution to this problem. Oxidase and alkali phosphatase

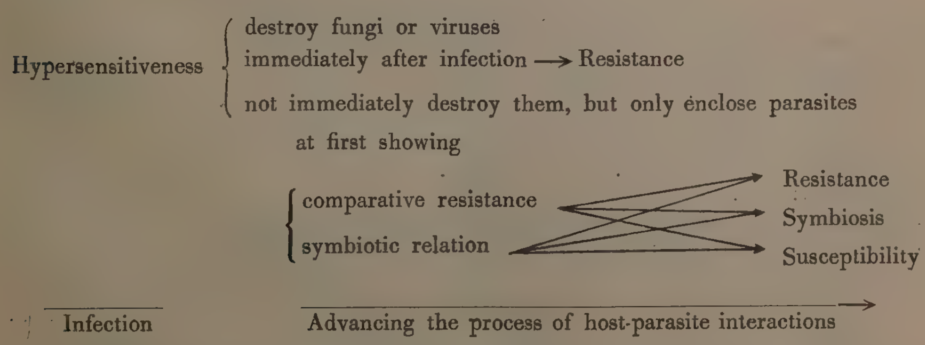
activities were also demonstrated to be positive on the bodies, as have been observed on the fungal diseases.

5. Discussion and conclusion

ALLEN (1) has reported marked increase in respiration in the diseased tissues and that most of it occurs in the noninvaded regions. He has also clearly demonstrated that the increase is due to uncoupling respiration from oxidative phosphorylation and a toxic metabolite derived from fungi may act as an uncoupler as well as dinitrophenol.

The author's finding that oxidase system, for example as measured by Nadi reaction, would increase upon the sites of hypersensitive regions which had developed by the invasion of fungi or viruses, may indicate a shifts in oxidative relations, being in accord with the results of experiments concerning the increased respiration in the affected tissues (22, 23). Besides this, of interest is the fact that increased phosphatase activity and, moreover, deep staining reactions showing the presence of nucleic acid, both indicating the increase in phosphorus turn over, were observed on the same regions. Accordingly, the author has reason to believe that the formation of host hypersensitiveness, at least that of its first step, appears to be a product due to normal oxidation process, namely, to the oxidative phosphorylation. Thus the increased respiration in these tissues must be attributed, as a rule, to a coupling one (2).

The hypersensitiveness, unless associated with so-called anti-fungal action, does not seem to be effective for determining host resistance. The author has considered the inclusion bodies as a product of host hypersensitive response. Despite of this, no evidence that hosts are now resisting to viruses can be demonstrable. In other words, host hypersensitiveness in this case may be a symbiotic one, possessing viruses within themselves, namely within their mitochondria or microsome which come in contact or is newly formed around the infection as the product of that reaction. Hypersensitiveness, therefore, will only suggest that host-parasite relationship is developing comparative-resistantly, and whether actually it resist or not depends upon the further response of the interaction. If showing by a schematic representation, the relation would be such as the following:



Susceptibility will be a phenomenon not forming hypersensitive reaction or when if it is formed, a destruction of the reaction during the course of the interaction. For instance, in a later stage of virus infection, it has been ordinarily shown that the inclusion bodies

rupture into many of the small granules and the crystalline inclusions, presumably viruses themselves, are pouring out abundantly from within the bodies (12).

In conclusion, insofar as the author is aware, it appears that browning necrosis or hypersensitive response which had previously been supposed to show the host resistance, does not completely explain its mechanism. It is not the phenomenon which forms necrosis or hypersensitive regions, but is the actually resistant action that we are now questionable. The action may be, in some case, attributed to the anti-fungal substance, or in another, the deficiency of nutrient materials available to the parasite. Yet, as far as the resistance is the response between host-parasite relationship, we must look for their mechanism into the mutual relations, and not solely into host plants without fungi or fungi in vitro. Studies on the dynamic process of that interaction which would appear upon the locus of parasitic invasion may be provide an objective approach for the possible solution to the basic mechanism of resistance in plants.

The author takes great pleasure in expressing his gratitude to Emeritus Prof. T. HEMMI and Prof. S. AKAI, Kyoto University, for their generous guidances and encouragements throughout the investigation.

References cited

1. ALLEN, P. J.: Phytopath. 43: 221–229, 1953.
2. AKAZAWA, T. and I. URITANI: Jour. Agr. Chem. Soc. Japan 29: 381–386, 1955.
3. BLACK, L. M., C. MORGAN, and R. W. G. WYCKOFF: Proc. Soc. Exp. Biol. & Med. 73: 119–122, 1950.
4. DUFRÉNOY, J.: Amer. Jour. Bot. 23: 70–79, 1936.
5. HIRAI, T.: Forsch. Gebiet PflKrank., Kyoto 4: 133–137, 1951.
6. ———, H. GOTO, H. KATO, and T. YASUMI: Ann. Phytopath. Soc. Japan 16: 1–5, 1952.
7. ——— and M. OKADA: Tohoku Natl. Agr. Exp. Sta. Report. (in press)
8. ———: Reading at the Ann. Meeting Phytopath. Soc. Japan, Tokyo, 1955.
9. ——— and M. OKADA: Tohoku Natl. Agr. Exp. Sta. Report. (in press)
10. ——— and Y. SATO: Ibid. (in press).
11. ———, Y. KOSHIMIZU, and T. KOIWA: Virus (Japan) 2: 257–262, 1952.
12. ——— and ———: (not publication)
13. HODGSON, R., W. H. PETERSON, and A. J. RIKER: Phytopath. 39: 47–62, 1949.
14. KOKUBUN, K., M. YAMAMOTO, and T. HIRAI: Tohou Agric. 5: 261–263, 1951.
15. ——— and T. HIRAI: Tohoku Natl. Agr. Exp. Sta. Report. (in press).
16. KOSHIMIZU, Y. and T. HIRAI: Ann. Phytopath. Soc. Japan 15: 21–25, 1950.
17. MATSUO, K., T. NOMURA, and F. IWAKIRI: Experiments on the control of snow-damages in agricultural plants. Minist. Agric. & Comm., pp. 108, 1944.
18. PLATTNER, P. A. and N. CLAUSSON-KAAS: Helv. Chim. Acta 28: 188–195, 1945
19. SAKAMOTO, S.: Sci. Rep. Res. Inst. Tohoku Univ. D–1,2: 15–23, 1951.
20. SCARTH, G. W.: Plant Physiol. 14: 129–143, 1939.
21. SUZUKI, N.: Biol. Science (Japan) 2: 117–128, 1950.
22. ———: Jubilee Pub. Prof. Tochinai & Prof. Fukushi, 229–235, 1955.
23. URITANI, I. and S. TAKITA: Jour. Agr. Chem. Soc. Japan 27: 168–174, 1953.

Explanation of Plates

Histo- and cytochemical observations upon the tissues affected with the snow-blight fungus, *Typhula incarnata*. Epidermal cells from inner side of leaf-sheath of wheat plants were mostly used.

Plate I.

1. Fuchsin-SO_3 reaction. Note positive to browning cell into which the fungus penetrate.
2. Millon reaction. Positive to cell end adjacent to affected cell.

3. Sudan III staining. Plasm granules around the infection take up well dye.
4. Ditto. The infection hyphae are seen to be enclosed by the plasm granules which are stained with dye.
5. Molish's reaction for sugars. One end of the noninvaded cell (appears upper) adjacent to the affected (appears lower) shows positive reaction.
6. Ditto. Positive to the sites of infection. Note plasm granules come in contact around the infection point.
7. Ditto., presumably microsomes in a healthy cell (appear upper left) are moving towards the affected (lower right). Note positive to cell wall.
8. Seliwanoff's reaction for sugars. Positive to plasm granules around the infection as well as to cell walls.

1–8 ca. × 400

Plate II.

9. Diazo reaction, no clearly positive reaction in the affected cells (which appear lower and rignt) or healthy cells adjacent to the former.
10. Ehrlich's indole reaction. Positive to cell wall and to the plasm adjacent to the wall.
11. Deep staining cells existing near the infecion, show alkali reaction. Stained with pH indicator dyes.
12. Ditto.
13. Oil drops appearing in the apparently healthy cells around the infection.
14. Ditto., treated with alkali.
15. Nadi reaction. Microsomes to which the reation are positive, are moving towards the affected cell (the latter appears upper right).
16. Ditto., microsomes are coming in contact to browning cell (which appears upper right).

9–11 ×400, 12 ×70, 13–14 ×500, 15–16 ×750

Plate III.

17. Alkali phosphatase reaction. Fungus large granules in affected cells which will appear when host resists to fungi.
18. Infection sites stained with azur I.
19. Alkali phosphatase, fungus large granules and the positive reaction to cell end (appears right).
20. Ditto., hyphae penetrating within cells and fungus large granules.
21. Plasm granules around the infection stained with thionin.
22. Ditto.
23. Alkali phosphatase, positive to the browning tissues (appear left).
24. Schiff reaction, positive to the browning tissue in leaves (appears upper part).

17 ×400, 18–22 ×600, 23 ×45, 24 ×300

Plate IV.

25. Alkali phosphatase, plasm granules to which the reaction is positive, are moving towards the cell end adjacent to the affected (the affected appears left).
26. Ditto., positive to nucleus in cells near the affected cells.
27. Ditto. Strongly positive to a cell (appears left) which is beginning to show browning.
28. Ditto., positive to plasm granules around the infection.
29. Plasm granules in a healthy cell which show a gradual moving to the affected (the affected appears right). Stained with thionin.
30. Ditto., although ribonuclease pretreated. Note the plasm is not stained.
31. The same as figure 29.
32. The same as figure 30.

25, 27 ×600, 26, 28, 29 ×800, 30, 32 ×700, 31 ×500

Plate I

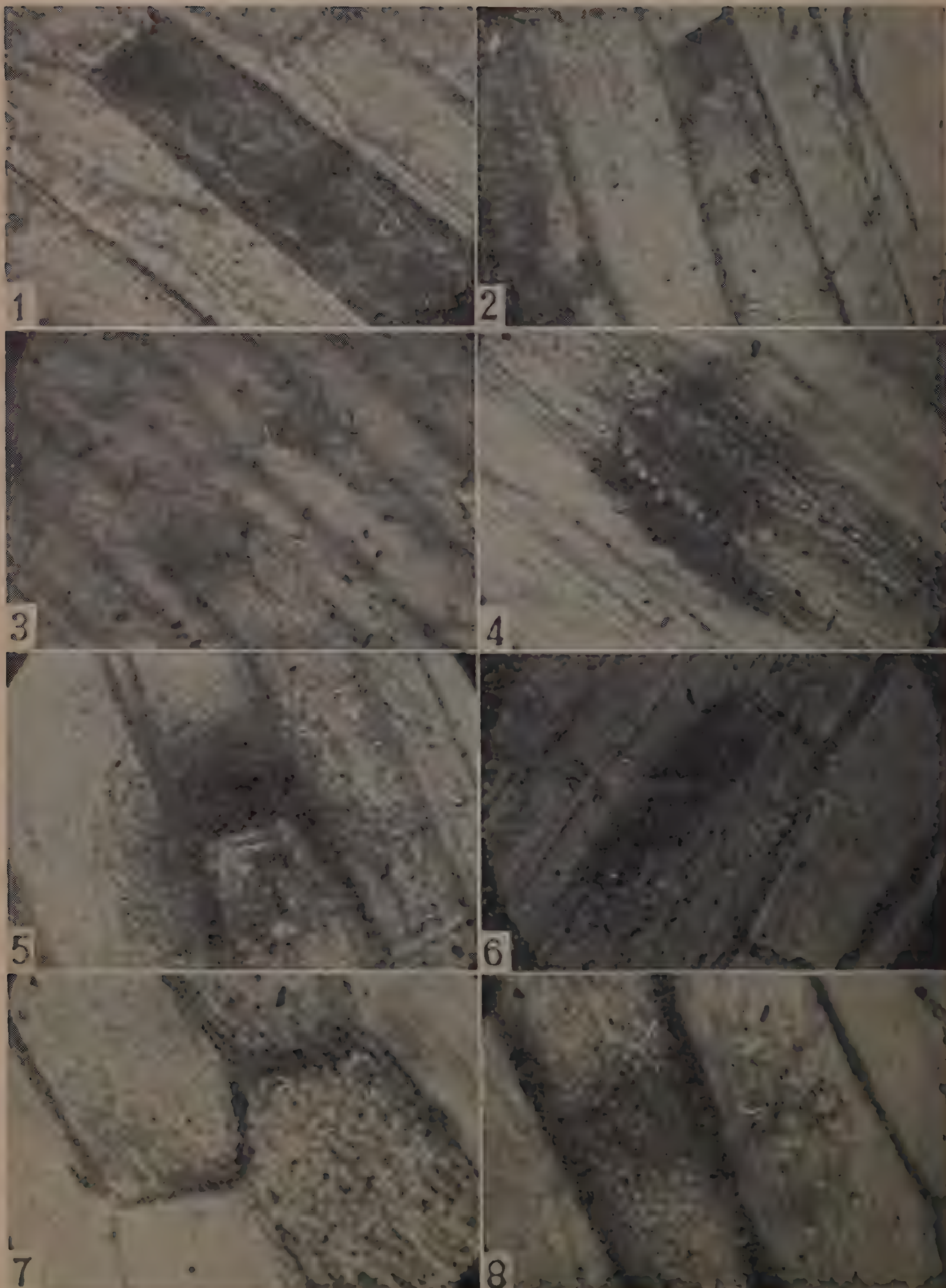


Plate II

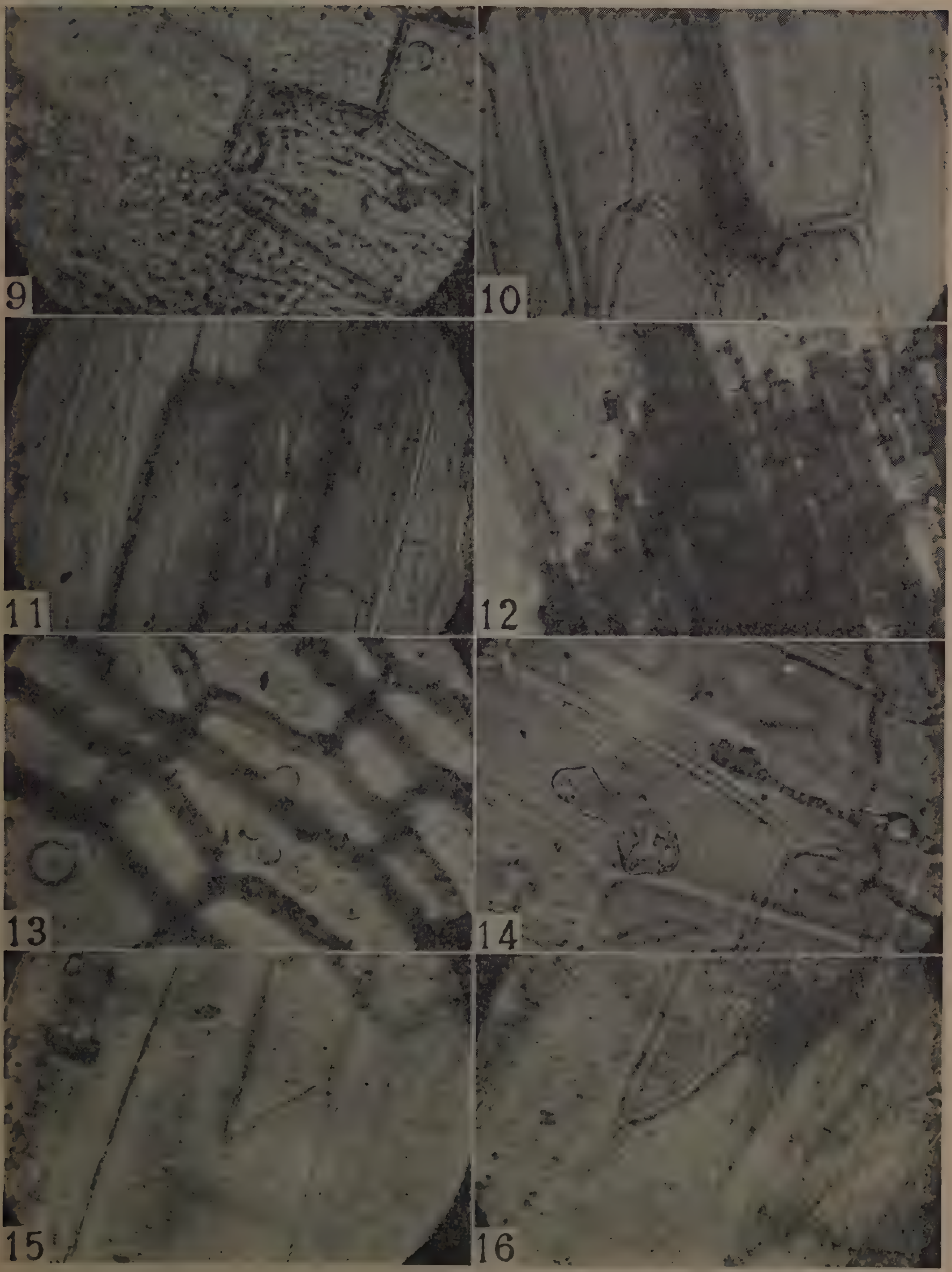


Plate III

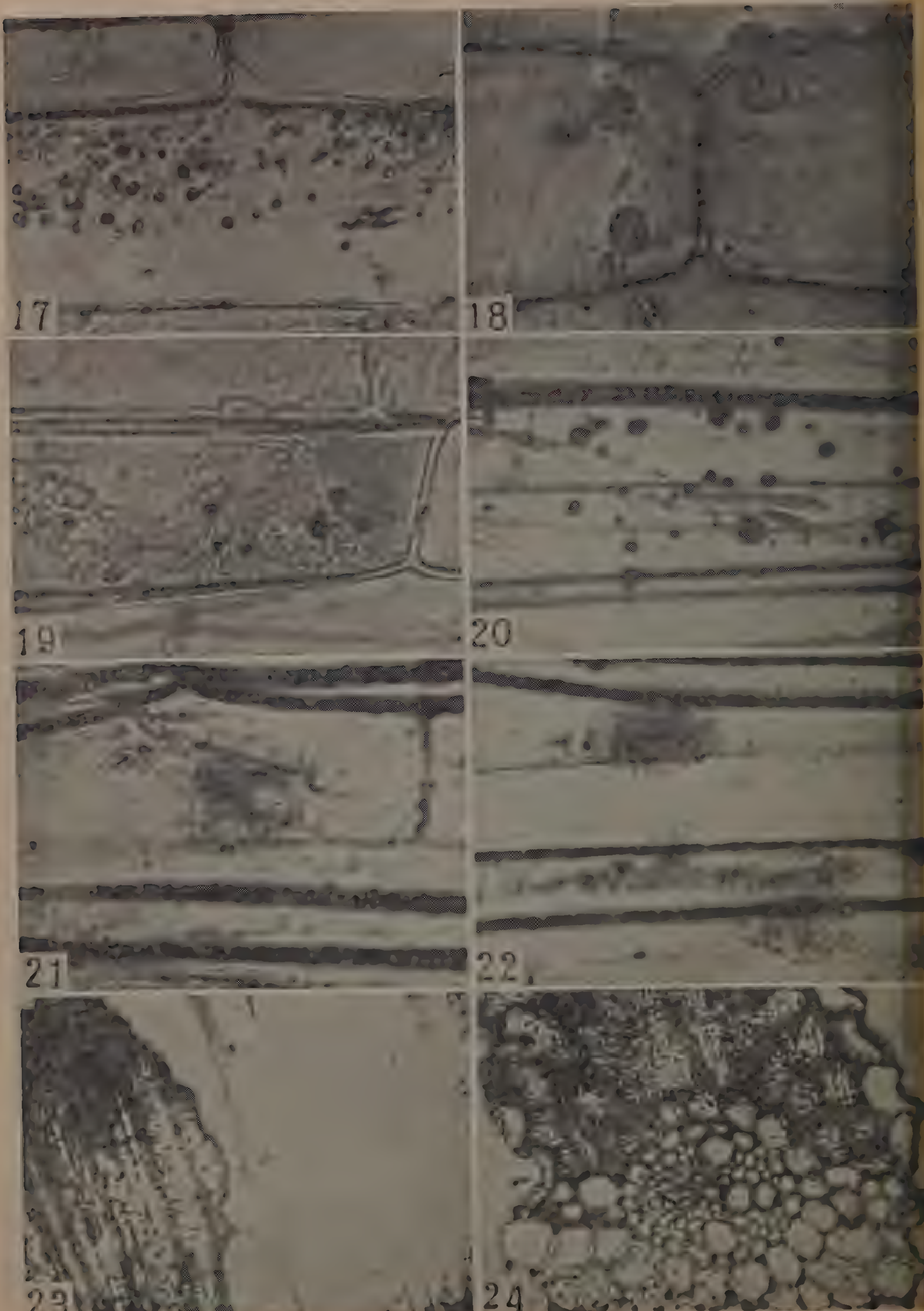
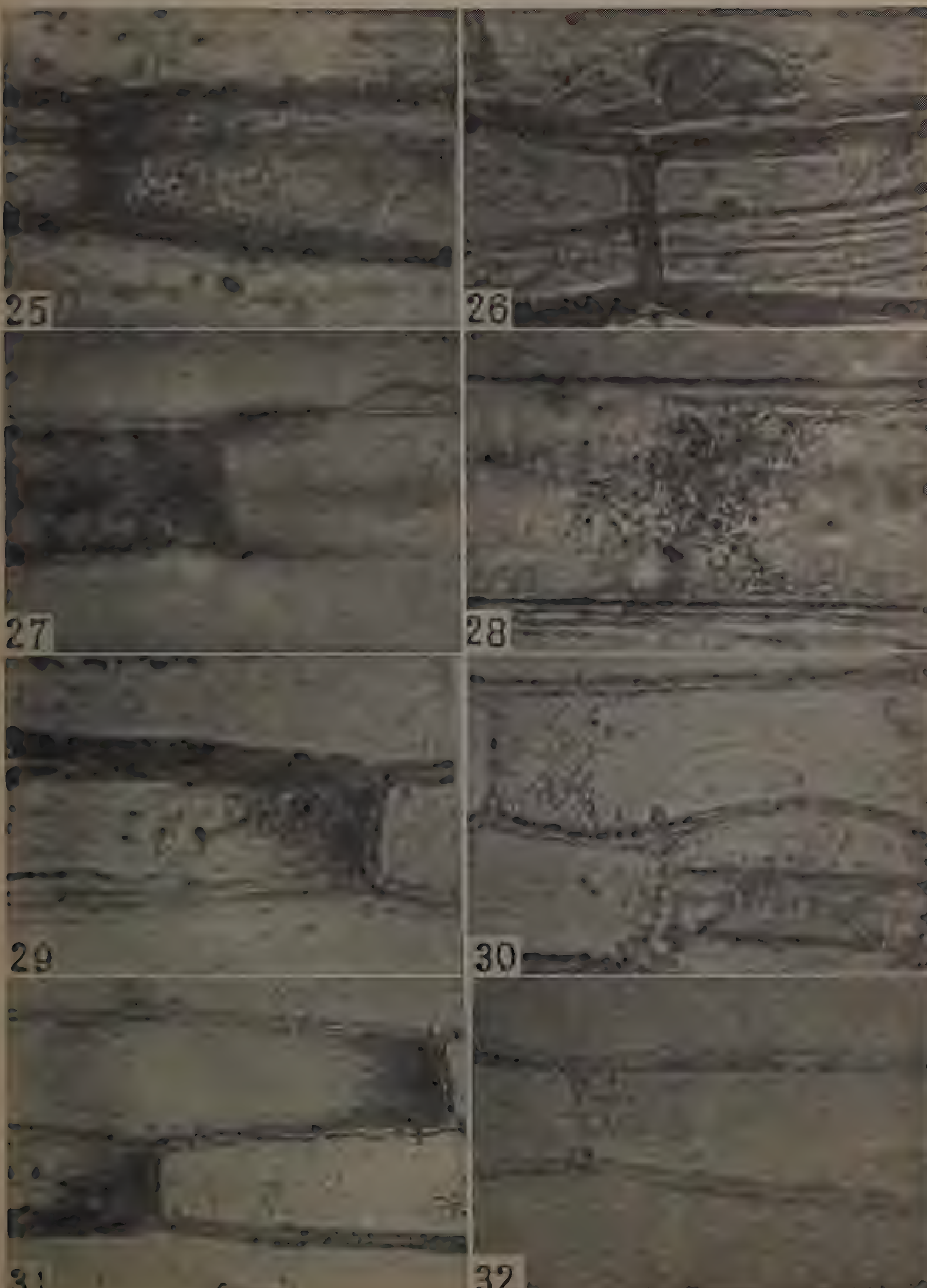
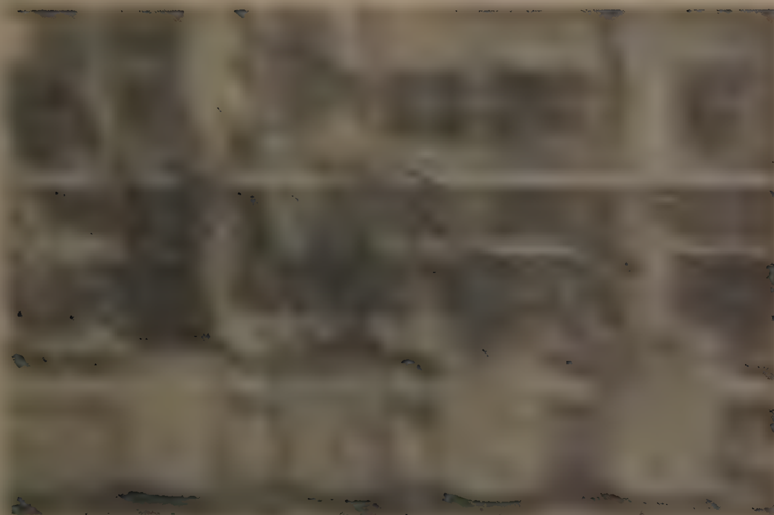


Plate IV





に濾紙を入れてつくつた湿室中で，28°C 下で発芽せしめた．結果は次のようである．

第1表 Na-pentachlorophenate の水稲種籾発芽に及ぼす影響

Effect of sodium pentachlorophenate upon the germination of rice seeds.

Concentration ppm	After 4 days		After 5 days	
	Number of seeds germinated	Per cent germination	Number of seeds germinated	Per cent germination
Control	20	100	20	100
100	20	100	20	100
200	11	55	11	55
1000	0	0	0	0

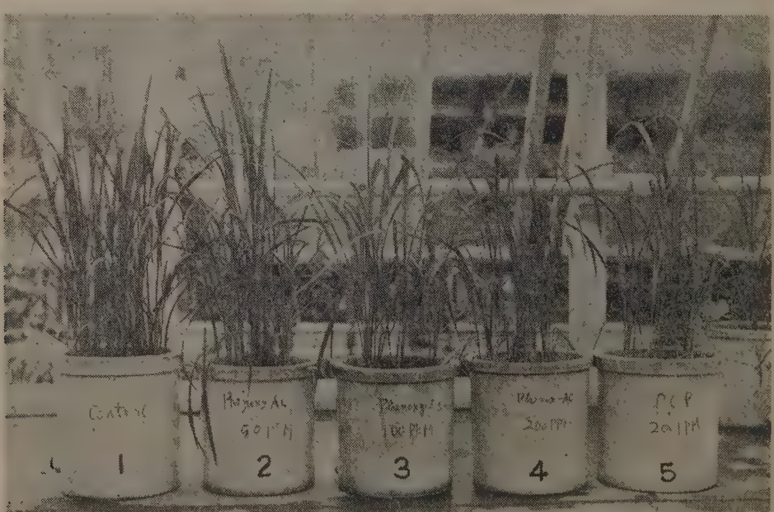
以上のように，0.01% (100 ppm) の薬液に浸漬した場合には，発芽は control と同じく 100% を示すが，芽の発育は可成り遅れるようである (第1図)．しかし 6 日後には芽も可なり伸長するので，著しい薬害の徴はあらわれない．しかるに 0.1% (1000 ppm) 液では薬害著しく，種籾は全然発芽しない (第1図)．

次に上記の薬液を水稲の根を通して吸収せしめた場合の薬害を調査した．ポットに育成した稲苗 (京都旭種) が長さ約 50cm に達したときに，ポット中の土壌及び水の重量に対して 0.02, 0.01, 0.005% になるように，pentachlorophenol, Na-pentachlorophenate, pentachlorophenoxyacetic acid を加えて，5日及び15日後にその薬害程度を調査した．結果は第2表及び第2,3図の通りであるが，水溶性の高い Na-pentachlorophenate の薬害が最も甚しく，0.005% (50 ppm) で既に可成りの程度に薬害を示した．而して pentachlorophenoxyacetic acid が最も薬害が少い．pentachlorophenol 及び pentachlorophenoxyacetic acid の 0.005% は外見上殆んど薬害を示さない．

第2表 Pentachlorophenol 化合物の水稲苗に対する薬害

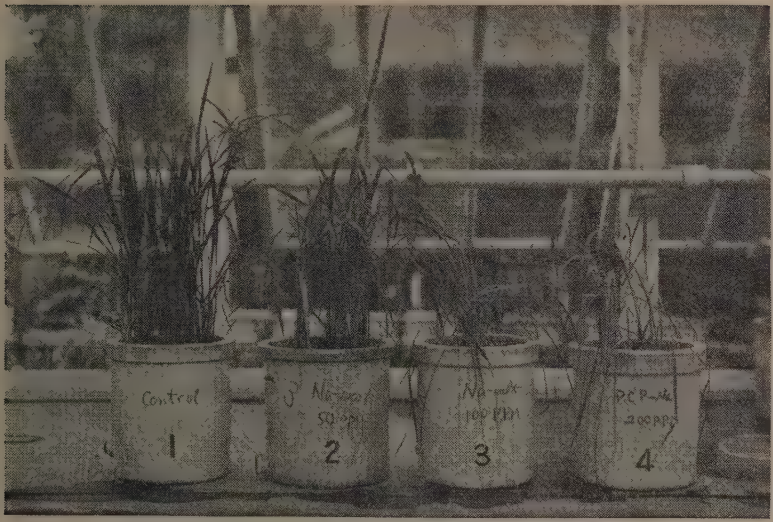
Injury of pentachlorophenol compounds to rice seedlings.

Fungicides	Concentration ppm	Degree of damage	
		After 5 days	After 15 days
Na-pentachlorophenate	200	+++	dead
	100	+++	dead
	50	++	plants became yellow and about the half of plants died
Pentachlorophenoxyacetic acid	200	++	dead
	100	+	plants became yellow
	50	±	almost no change
Pentachlorophenol	200	++	dead
	100	++	plant became yellow and about the half of plants died
	50	±	almost no change



第2図 Pentachlorophenol 化合物の水稲に及ぼす影響 (July, 1952)
Injury of pentachlorophenol compounds to rice seedlings.

1. Control
2. Pentachlorophenoxyacetic acid 50 ppm
3. 〃 100 ppm
4. 〃 200 ppm
5. Pentachlorophenol 200 ppm



第 3 図 Na-pentachlorophenate の水稲苗に及ぼす影響 (July, 1952)
Injury of sodium pentachlorophenate to rice seedlings.
1. Control
2. Na-pentachlorophenate 50 ppm
3. 〃 100 ppm
4. 〃 200 ppm

2. 薬剤を根から吸収せしめた場合の水稲胡麻葉枯病感受性 CROWDY & WAIN(3) は trichlorophenoxyacetic acid 等の phenoxycarboxylic acid を pot 植のソラマメに与えて，チョコレート斑点病に対する影響を見ているが，それ等の化合物を根から吸収せしめ，或は撒布して，発病をかなり抑えている．氏等は，これらの化学構造が 2,4-D や indolacetic acid 等の植物生長ホルモンと似ているので，植物体内を移動し易いのであろうと考えている．

筆者等は 1 : 50000反 pot に生育せしめた水稲が約 60cm に達したとき，pot 内容（土壌及び水）に対して pentachlorophenol 及び pentachlorophenoxyacetic acid を 50 ppm，Na-pentachlorophenate を 25 ppm の割合に施して，その後の稲の病害感受性変化を調査した．接種試験は施薬後 5 日目に行つた．即ち胡麻葉枯病菌 No. 13 の分生胞子を 1% sucrose 溶液中に懸濁して，80 倍顕微鏡 1 視野中に約 5 個の濃度とし，各 pot 20ml 宛噴霧した．噴霧後 3 日間 30°C の湿室に保つて，その後温室に移した．

第 3 表 水稲の胡麻葉枯病感染に及ぼす pentachlorophenol 化合物の影響
Effect of pentachlorophenols absorbed upon the infection of *Helminthosporium* of rice p'ants.

Fungicide	Concentration ppm	Number of leaves used	Average length of leaf	Number of spots per 10cm length of leaf		Per cent* r duction
				large	Total	
Pentachlorophenol	50	102	55.1	0.02	0.57	91
Pentachlorophenoxyacetic acid	50	85	53.9	0.10	1.23	80
Na-pentachlorophenate	25	57	54.1	0.01	0.78	87
Na-pentachlorophenate**	50	76	55.5	0.09	1.67	72
Control	—	151	51.5	0.42	6.00	0

* Significant in 5 % level.

** Sprayed 0.005% solution upon leaves before 24 hours of the inoculation. 100 ml of the solution was added with 0.6 gr of mixture of Casein 2 and lime 7.

発病程度の測定にあたつて，まず病斑を大・中・小と3別して，それ等の数を測定した．即ち病斑の大さが5mm以上あつて，壊死部及び黄化中毒部の認められるものを大，壊死部，黄化部は認められるが，その直径2-5mmのものを中，極めて小さい壊死部のみで，黄化部が認められない点状のもの（直径1mm以下）を小として計算した．而してこれ等病原菌の侵入に対する薬剤の影響のほかに，薬剤が病状の進展に対して示す影響をも当然考慮しなければならない．筆者等は病斑の大さを測定しなかつたので，小病斑が大病斑に拡大進展する比率を計算した．結果は第3及び4表に示す通りである．

第4表 Pentachlorophenol 化合物の水稲薬の胡麻葉枯病々状進展に及ぼす影響
Influence of pentachlorophenols to the development of lesions of Helminthosporiose in rice seedling.

Fungicides	Concentration ppm	Index of spot enlargement	Per cent inhibition of spot enlargement
Pentachlorophenol	50	3.5	64
Pentachlorophenoxyacetic acid	50	8.1	-16
Na-pentachlorophenate	25	1.3	81
Na-pentachlorophenate*	50	5.4	23
Control	—	7.0	0

* see Table 3 **

上表の結果のように，薬剤を土壌に施用した場合には．水稲は著しく発病を減少した．特に pentachlorophenol 及びその sodium 塩を使用した場合に効果が高い．而して病原菌が侵入した後，病斑が伸展する状況を見るに Na-塩を施用した場合は多少病斑の拡大を抑制するようであるが，他の薬剤では病斑拡大を抑える力が少いようである．尚 Na-pentachlorophenate 25ppm を与えたものでは，薬害が著しく，葉の黄化したものが多かつた．Na-塩 0.005% 液を撒布した場合にも葉面に水浸状斑点を点々と生ずるが，この場合にも病斑の拡大を抑制する力はない．上述のように，pentachlorophenol 及びその Na-塩を施用した場合には発病を抑え，且つ病斑の拡大を抑制したが，pentachlorophenoxyacetic acid を施用した場合には，感染は抑制せられるが，病斑の拡大は抑制せられないようである．

3. 考察

薬剤を内科的に使用して，疾病を予防又は治療しようという所謂化学療法は，現在抗生物質と共に植物治病学上の一つの焦点にちるといえよう．このような chemotherapy に際しては，寄主植物内に導入された薬剤そのものが直接病原菌に作用する，所謂 systemic fungicide として働く場合と，寄主体内でおこる変化によつて効果をあらわす場合とが考えられる．

筆者等は pentachlorophenol 化合物を水稲に施用して，胡麻葉枯病の発生抑制に効果を収めたが，稲体内に於ての作用機作に関しては尚明かにしていない．Keyworth & Dimond(6) はトマトの根に薬害を与えることが *Fusarium* による萎凋病感受性を減ずる1原因と述べている．しかしこのようなことをすべての疾病にあてはめて考えることは難しい．氏等は根処理によつて炭水化物含量を変ずることを述べているが，一般に 2,4-D などによつて含水炭素の増加が報告されている．筆者等は Na-pentachlorophenate を吸収せしめた小麦葉中の還元糖の測定を試みたが，標準区との間に明かな差を認めていない．

Stubbs(10) は tomato を砂耕培養して，それに 0.05% になるように gliseofulvin を与えた場合に *Alternaria solani* による輪紋病を 99～100% 防除し得ると報じた．又 Brian(2) は gliseofulvin を小麦に与えると，その guttation drop が強い殺菌作用を呈することを述べている．これらのことはこの物質が植物の組織内を移動した事を証するものであろう．

筆者等は Pentachlorophenol 化合物を吸収せしめた水稲に於ける機作について更に研究をすすめる考えである．

4. 摘要

本報告に於ては，pentachlorophenol, sodium pentachlorophenate 及び pentachlorophenoxyacetic acid の水稲胡麻葉枯病感受性に及ぼす影響を論述した．

1. 0.1% Na-pentachlorophenate は稲種籾の発芽を完全に抑止する．0.01% では芽の発芽は遅れるが，発芽は抑制せられない．而して苗に対する薬害は 0.005% に於て尚著しく，約半数の苗は枯死に至る．

2. Pentachlorophenol 及び Pentachlorophenoxyacetic acid の薬害は曹達塩より稍々弱く，0.005% では外見上殆んど薬害の徴を呈さない．

3. Pentachlorophenol 50ppm, pentachlorophenoxyacetic acid 50ppm, sodium pentachlorophenate 25ppm を土壌を通して水稲苗に吸収せしめた場合に，3薬剤共胡麻葉枯病の感染を著しく減少せしめたが，形成せられた病斑の伸展には余り抑制力をもつていない．而して sodium pentachlorophenate は感染に対しても，亦病斑伸展抑制にも供試薬中最も効果が高かつたが，尚多少の薬害を示した．

引 用 文 献

1. 赤井重恭・上山昭則・達山和紀：植物病害研究 5(1)：33-35, 1954.
2. Brian, P.W.: Ann. Appl. Biol. 39: 434-438, 1952.
3. Crowdy, S.H. & R.L. Wain: Ibid. 38: 318-333, 1951.
4. Davis, D.: Phytopath. (Abst.) 42: 465, 1952.
5. 福島 茂：日本植病学会報 11 (4)：162-171, 1947.
6. Keyworth, W. G. & A. E. Dimond: Phytopath. 42: 311-315, 1952.
7. 西門義一・大島俊一・森田日出男：農学研究 39(4): 1-5, 1951.
8. ———, ———, ———: Ibid. 40(1): 7-12, 1951.
9. 奥八 郎：植物病害研究 5 (2)：77-82, 1955.
10. Stubbs. J.: Ann. appl. Biol. 39: 439-441, 1952.

Résumé

In the present report the writers reported the result of experiments on the effect of pentachlorophenol, sodium pentachlorophenate and pentachlorophenoxyacetic acid upon the susceptibility of rice plants to Helminthosporium leaf spot.

1. Rice seeds were inhibited in their germination completely in 0.1 % sodium pentachlorophenate, but not in 0.01 per cent. They grew very slowly in the latter solution. The injury to seedlings of sodium salt, however, was severe even in 0.005 per cent solution, and about half of seedlings died.

2. The injury to seedlings of pentachlorophenol and pentachlorophenoxyacetic acid was less severe than sodium salt of pentachlorophenol. They show almost no injury in appearance in 0.005 per cent solution.

3. The infection of the present disease was checked greatly, if pentachlorophenol and pentachlorophenoxyacetic acid were given in 50 ppm, and sodium pentachlorophenate in 25 ppm per pot and absorbed by the seedlings through soil. The area of the necrotic lesions did not reduced by the application of the former two chlorophenols. Sodium pentachlorophenol demonstrated the most effective control in the infection and also in the enlargement of spot area. The slight iujury, however, was also found in the seedlings treated with sodium salt in the concentration of 25 ppm.

稲胡麻葉枯病菌の発育に及ぼす炭素源の影響に就いて*

田　中　寛　康

Hiroyasu TANAKA: On the influence of carbon sources upon the growth of *Cochliobolus miyabeanus.*

1. 緒　　言

植物病原菌とその寄主との間の相互関係を考察するに当り，まず病原菌及び寄主植物の夫々独自の根本的な生理作用を追求することは重要なことである．寄主植物である高等植物の生理作用に関してはかなり研究も多いが，病原菌に関しては尚定量的に取扱われたものが比較的少ない．稲の重要病原菌についてみるに，稲熱病菌に就いては炭素源代謝，窒素源代謝が，田中・香月・香月(15)，富沢(19)，大谷(10)，栃内・中野(18) 等によつて研究されているが，胡麻葉枯病菌に就いては，西門(9)，田中・依田(17) 等の報告があるに過ぎない．筆者は稲胡麻葉枯病菌 (*Cochliobolus miyabeanus*) の炭素源利用に関して，2, 3 の観察を行つたので，その結果を報告する．本稿を草するに当り，実験中始終懇篤なる御指導を恭うした赤井重恭教授，並に種々懇切な助言を賜つた研究室員諸氏に対して謹んで感謝の意を表する．

2. 実験材料及び実験方法

1. 実験材料　供試菌として研究室保存の胡麻葉枯病菌 13 号を使用した．2% 蔗糖加用馬鈴薯煎汁寒天培養基上の菌令 15 日の菌叢を実験に供した．培養基としては Czapek 液(14) を用い，その sucrose を種々の炭素源で置換えた．而してこれらの炭素源の炭素量が Czapek 液 1l に 8 gr の割合になるように加えた(7)．即ち glycerin は 20.45 gr，monosaccharide は 20 gr，disaccharide は 19 gr，polysaccharide は 18 gr である．

2. 実験方法　固体培養基上での発育試験には，直径約 8 cm のペトリー皿を常法により滅菌した後，上記培養基 (2% 寒天加用) を注加，冷却後その中央に供試菌を接種し，28°C の定温器中に保つて 2 日毎に菌叢直径を測定した．

液体培養試験には容量 100 ml の三角罎を使用した．上記培養液を 15 ml 宛分注し，コッホ殺菌釜で 30 分ずつ 3 日間間歇滅菌した後，菌を接種し，28°C の定温器中に保つて 5 日毎に 20 日間乾燥菌体重を測定した．尚供試菌による炭素源の消費量は上記発育試験に於ける菌体濾液に就いて測定した．即ち monosaccharide では菌体濾液をそのまゝ disaccharide では菌体濾液を加水分解した後，LEHMANN-MAQUENNE-SCHOORL 法により残つた炭素源の量を定量し，後者では測定値に 0.95 を乗じた量を培養基に加えた炭素源の量から差引いて消費量を求めた．而して炭素源の経済率は，(乾燥菌体重/消費した炭素源の量) ×100 なる式により算出した．液体培養基上に於ける菌の発育に及ぼす炭素源濃度及び培養温度に関する実験には，炭素源として glucose のみを用い，容量 150 ml の三角罎に上記 Czapek 液を 25 ml 宛入れ，実験を行つた．

3. 実験結果

1. 固体培養基上の菌糸発育に及ぼす炭素源の影響　上記実験方法により，菌糸の発育と炭素源との関係を求めた (第 1 表)．

第 1 表　異る炭素源を含む Czapek 寒天培養基上の稲胡麻葉枯病菌の菌叢直径

Diameter* of mycelial mats of *Cochliobolus miyabeanus* grown on Czapek's agar media containing different carbon sources.
All the carbon sources were added at the rate of 8 gr of carbon per liter. (March, 12-20, 1953).

Carbon sources	culture duration (day)			
	2	4	6	8
Glycerin	18.8	39.8	59.8	77.0
Xylose	17.3	45 3	71.3	(84.5)
Glucose	18.3	38.5	72.8	(83.3)
Fructose **	16.3	43.3	70.3	(83.7)
Mannose**	16.7	43.0	73.0	(82.7)
Galactose**	17.3	45.0	73.7	(84.0)
Sucrose **	18.0	44.7	74.3	(84.3)
Maltose **	19.3	43.7	74.7	(84.3)
Lactose	18.3	40.0	63.8	80.3
Soluble starch **	14.7	36.7	64.3	82.3

* Average of four Petri dishes,
** of three Petri dishes.

Number in parenthesis is the diameter of mycelial mat, which expanded to the edge of Petridish.

* 京都大学農学部植物病理学研究室業績，第 80 号　稲胡麻葉枯病に関する研究，第 18 報

その結果によると，菌糸がペトリー皿一杯に拡がるのは xylose, glucose, fructose, mannose, galactose, sucrose, maltose では 8 日目，glycerin, lactose, sol. starch では10日目であつて，菌糸がペトリー皿一杯に拡がる日数によつて炭素源を 2 つの群に分けることが出来る．而して 8 日目の測定値を除いた他の結果全体に就いては，1% 水準で有意差が認められたが，しかし各炭素源の平均値では，どの 2 つの間にも有意差は認められていない．

次に菌糸の着色状態を比較した (第 2 表)． 着色部分には夫々相当量の胞子が形成されていたが，必ずしも着色部分の総てに胞子が形成されているとは限らない． 菌糸の着色部を透過光線により撮影比較した結果は第 1 図の通りである．

Glycerin は着色状態悪く胞子形成も亦極めて悪いので，測定不可能であつた．培養開始後 2 日では各炭素源共殆んど着色せず， 3 日目頃から着色し始めるものと思われる． lactose は特に培養初期に於て，sol. starch は全期を通じて概して着色状態が悪い．

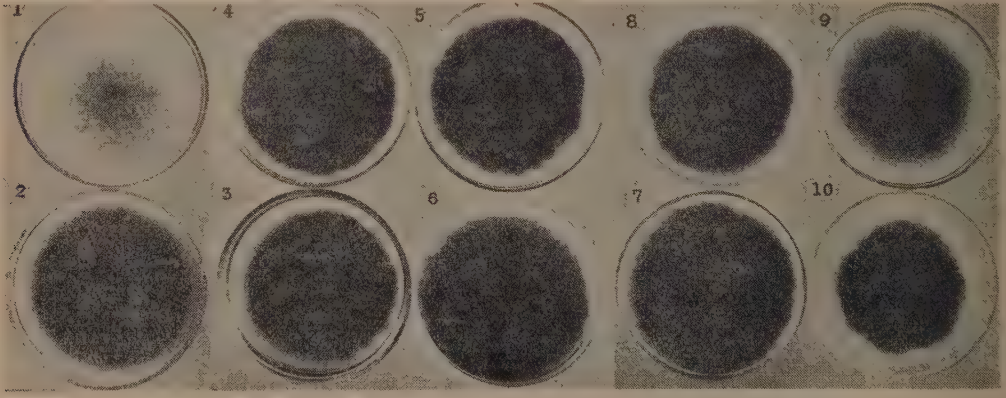
以上の結果から，胡麻葉枯病菌の栄養として，使用した炭素源中良好なものは (1) xylose, glucose, fructose, mannose, galactose, sucrose, maltose であつて，次は (2) lactose, sol. starch であり，(3) glycerin が最も悪い．

第 2 表 異る炭素源を含んだ Czapek 寒天培養基上に発育した稲胡麻葉枯病菌菌叢の着色部直径

Diameter of colored part in the mycelial mats of *Cochliobolus miyabeanus* grown on Czapek's agar media containing different carbon sources.

Carbon sources		Culture duration (day)		
		4	6	8
Xylose		26.0	53.0	75.8
Glucose		23.8	48.5	70.3
Fructose	**	23.3	49.3	71.3
Mannose	**	21.3	49.3	72.0
Galactose	**	20.3	54.3	75.3
Sucrose	**	24.0	69.3	70.7
Maltose	**	23.0	46.0	71.7
Lactose		15.3	48.0	72.5
Soluble starch	**	17.0	42.7	65.3

* Average of four Petri dishes,
** of three Petri dishes.



第 1 図 異る炭素源を含んだ Czapek 寒天培養基上の稲胡麻葉枯病菌の発育

Mycelial growth of *Cochliobolus miyabeanus* on Czapek's agar media containing different carbon sources, (6 days), (Mach, 18, 1953). Photographed by transmited ray.

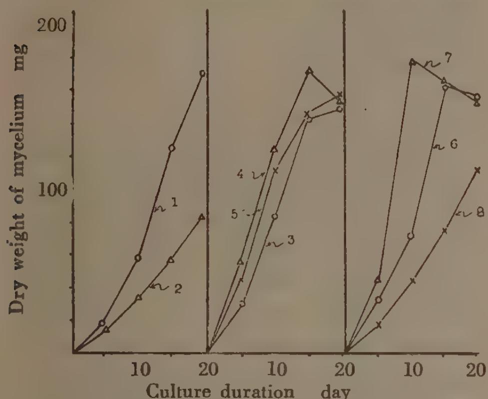
1 : glycerin, 2 : xylose, 3 : glucose. 4 : fructose, 5 : mannose, 6 : galactose, 7 : sucrose 8 : maltose, 9 : lactose, 10 : sol. starch.

2. 供試菌の液体培養基上の発育に及ぼす炭素源の影響．

供試菌の液体培養と各炭素源との関係は第 2 図の通りである．

実験は 2 回行つたが，その結果から結論されることは，(1) 乾燥菌体重の最大に達する日数は， maltose 10 日， fructose, sucrose 15 日，galactose 15 ～ 20日，xylose, glucose 20 日，arabinose 20 日以後である． (2) 乾燥菌体重の最大値は，maltose が最大であつて，xylose がこれに次ぎ， glucose, fructose, galactose, sucrose 等の順序であるが， これ等 4 者の間には大差がない． 而して lactose, arabinose が最少の値を示した． (3) 培養初期の

乾燥菌体重の増加状態. 5日目及び 10日目の乾燥菌体重の ½ の対数を求め, それらを平均した値によつて培養初期の乾燥菌体重の増加状態を調べた. その結果によると maltose > fructose, galactose > glucose, sucrose >



第 2 図　異る炭素源を含んだ Czapek 液に発育した稲胡麻葉枯病菌の発育と培養日数.

Mycelial growth of *Cochliobolus miyabeanus* grown on Czapek's culture media containing different carbon sources and the culture duration.
1: xylose, 2: arabinose, 3: glucose, 4: fructose, 5: galactose, 6: sucrose, 7: maltose, 8: lactose.

xylose > lactose > arabinose の順序である. この結果は多少の相異はあるが, (1) の結果と非常によく似ている.

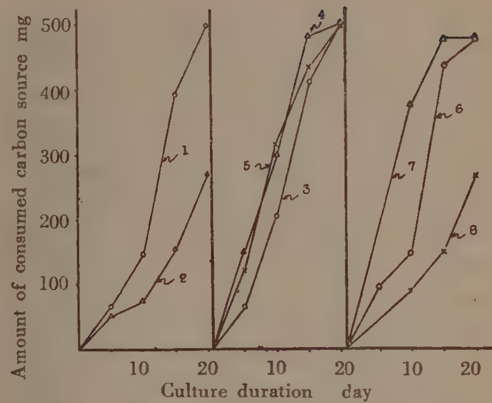
3. 液体培養基上の菌体による炭素源の消費.

乾燥菌体重秤量後の濾液を使用して, 上記実験方法によつて菌が消費した炭素源の量を測定した. 結果は第 3 図の通りである.

この結果からみると, maltose は 10～15 日の間に全部消費され, fructose, sucrose, galactose は 15 日頃に, xylose glucose は 15～20 日の間に消費し尽されるが, lactose と arabinose は 20 日以後に於てもかなり残つている. この結果は乾燥菌体重が最大に達する時期とよく一致している. 従つてこれらの事実から, 乾燥菌体重は炭素源の存在する間増加するが, 炭素源が消費し尽されると, 自己消化を始めるので乾燥菌体重が減少するものと考えられる.

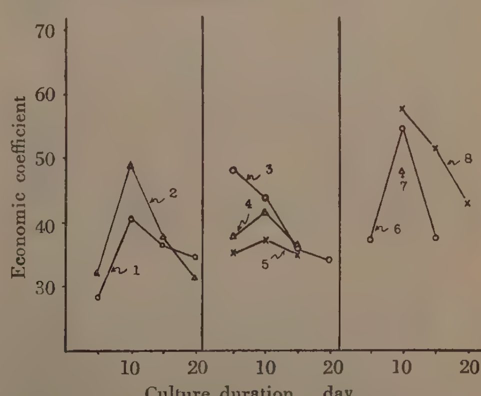
4. 液体培養基上の菌体によつて消費された炭素源の経済率.

上記計算法により各炭素源の経済率を計算した結果は第 4 図の通りであつて, 極く少数の例外はあるが殆んど総てが 10 日目に最高の値を示し, その後は減少の傾向を示している. 以上のことから本実験の範囲内に於いては, 10日目頃に炭素源の利用が最も能率よく行われるものということができる. 尚 10 日目の経済率に就いて検定した結果, 1 % 水準で炭素源間に有意差が認められた. 而して各炭素源の経済率の平均値間の差に就いて検定した結果では, 培養 10 日目の各炭素源の経済率は lactose > sucrose > arabinose > maltose > glucose > fructose > xylose, galactose の順に高い価を示し, xylose と galactose の間には有意差は認められなかつた. このこ



第 3 図　異る炭素源を含んだ Czapek 液上の稲胡麻葉枯病菌の炭素源消費と培養日数との関係

Relation of the culture duration to the carbon sources consumed by *Cochliobolus miyabeanus* grown on Czapek's culture media containing different carbon sources.
1: xylose, 2: arabinose, 3: glucose, 4: fructo·e, 5: galactose, 6: sucrose, 7: maltose 8: lactose.



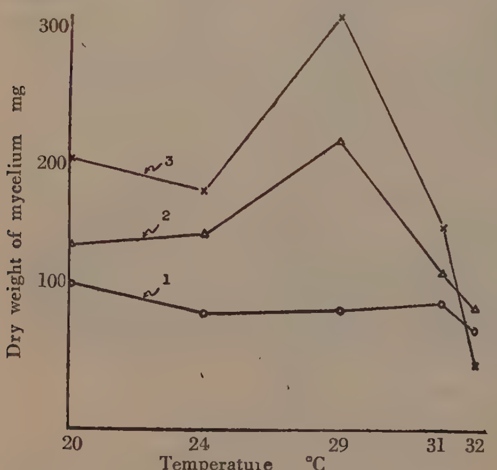
第 4 図　異る炭素源を含んだ Czapek 培養基上に発育した稲胡麻葉病菌の炭素源の経済率

Economic coefficient of carbon sources consumed by *Cochliobolus miyabeanus* grown on Czapek's culture media containing different carbon sources.
1: xylose, 2: arabinose, 3: glucose, 4: fructose, 5: galactose, 6: sucrose, 7: maltose, 8: lactose.

とから，disaccharide と monosaccharide の間には差がみられるようであるので，更に上記 8 種類の炭素源を pentose, hexose, disaccharide に分けて検定を行つた。その結果はそれらの間には有意差が認められた．而してそれらの平均値の差に就いては，pentose と disaccharide 間では 5% 水準で，又 hexose と disaccharide 間では 1% 水準で有意差があるが，pentose と hexose 間には有意差が認められなかつた。従つて monosaccharide と disaccharide の間には明かに差があつて，後者の方の経済率が大である。

5. 液体培養基上に於ける菌体の発育に及ぼす炭素源濃度並に培養温度の影響

培養基中に与えられた glucose の濃度と乾燥菌体重との関係実験 (2 回実験) 結果 (第 5 図) によると，実験の範囲内では glucose の濃度が大きい程乾燥菌体重が大となる．而して供試菌の発育に及ぼす培養温度の影響を 15 日間の培養結果で比較すると，glucose の濃度が 3 及び 5% に於いては，20〜24°C 間は大差なく，28°C に於て発育が最高に達し，更に温度が上昇するに従つて急激に低下する．しかし glucose が 1% の場合には，培養温度が低い程寧ろ乾燥菌体重が大となる傾向がある．これらに就いて検定を行つた結果では，glucose 濃度間及び培養温度間には夫々 1% 水準で有意差が認められ，更に



第 5 図　異る量の glucose を含む Czapek 培養基上の稲胡麻葉枯病菌が異る温度下で示す菌体乾燥重量

Dry weight of the mycelium of *Cochliobolus miyabeanus* grown on Czapek's culture media containing different amount of glucose under different temperatures.
1 :1%, 2 : 3%, 3 : 5%.
Average result of the two replications. After 15 days incubation.

glucose 濃度 1% と 3% 間及び 1% と 5% 間には 1% 水準，3% と 5% 間にはその平均値間に於いてのみ 5% 水準で有意差が認められた．而して培養温度 28°C と 20°, 24°, 32°C 間では夫々 1% 水準で，又 28°C と 31°C 及び 32°C と 20°, 24°C 間では 5% 水準で，31°C と 32°C 間では平均値間に於いてのみ 1% 水準で有意差が認められた．

4. 論　　議

以上の諸実験結果から，固体培養基上での稲胡麻葉枯病菌の発育には，炭素源として単糖類及び複糖類が良好であり，多糖類である可溶性澱粉は菌糸の伸長にはやゝ不良である。而でて多価 alcohol である glycerin は菌糸の伸長，分生胞子の形成に極めて不良のようである。栃内・中野(18) は稲熱病菌に対して可溶性澱粉並に glycerin を共に良好な炭素源としているが，富沢(19) によれば，glycerin はかなり劣り，大谷(10) の実験結果に於ても同様に不良である．これらの結果の相異は大谷(10) も指摘しているように，栃内等の実験は稲熱病菌の発育に必要な biotin や thiamin を添加していない為，正常な発育をなしえなかつたことによるものであらう．しかし胡麻葉枯病菌はその発育にこれらの要素を必要としないので，それらを添加しなくとも良好な発育を遂げる．且実験結果は富沢，大谷等の稲熱病菌に関する結果とよく類似している．

液体培養基上に於ける発育に関して，単糖類，複糖類中では，最高菌体重に達する日数，最高菌体重及び培養初期に於ける菌体重の増加速度等から，maltose が炭素源として最も良好であり，arabinose が最も不良，lactose がこれに次いで不良である．maltose が炭素源として最良であることは栃内・中野，富沢，大谷等も等しく認めているところである．一般に複糖類が多くの菌類によつて利用される時，先ず加水分解によつて単糖類に分れ，しかる後始めて吸収利用されることが知られている(7,12)．又六単糖の中では正常型よりも異常型即ち γ 態のものが呼吸材料として良好であることも知られているが(3)，呼吸材料として良好なものは，又菌の炭素源としても良好であるから，γ 態は菌によつて利用され易い．しかるに maltose は加水分解されて γ 態 glucose を生じ，又 sucrose も γ 態 fructose を生ずる．従つて maltose が加水分解によつて γ 態 glucose を生じ，これが菌によつて直に利用され，又分解すると考えるならば，水溶液中で正常型の glucose を生じている glucose 培地よりも maltose 培地の方が菌の生育に好適であることはうなづける．sucrose は分解せられて glucose と fructose を，lactose は glucose と galactose を生ずるが，この様な 2 種の炭素源の混在

は菌の種類によつて異ることは勿論であるが，菌の利用に有利に働く場合と不利に働く場合とがある(7). HORR(6)は *Aspergillus niger* が glucose と galactose の混合培地上で生育する時，相助的関係があることを見出した．STEINBERG(13)によると，*A. niger* の培養では D-mannitol-lactose は相助的関係にあるが，glycerol-D-galactose では逆の関係にあることを見出した．しかし MALGOLIN(8)は *Phycomyces blakesleeanus* や *Pythiomorpha gonapodyoides* 等では，炭素源の混合は全く加法的に働くことを認めた．本菌による lactose の利用が maltose や sucrose より劣るのは，glucose と galactose の混在が庇護作用により恐らく不利に働くものと考えられるが，又加水分解に関与する酵素，即ち lactase の活性が小であるとも考えられる．

次に単糖類に於いては， fructose が最も良好であり，glucose, galactose 等がこれに次ぎ，xylose, arabinose と不良となる．一般に微生物によつて，糖類は直接酸化されて pyruvate となり，しかる後 alcohol 醱酵，乳酸醱酵，又は TCA-cycle に従つて呼吸に用いられる(2,4). この時糖は先ず，enol 型になる．故に細胞によつて容易に吸収される glucose, fructose, mannose, galactose 等の所謂醱酵糖の中でも，容易に enol 型に変化する glucose, fructose, mannose は直接酸化的代謝に利用され易く，galactose は比較的利用され難い(12). xylose, arabinose 等は更に enol 型になり難い故に一層利用が困難なものと考えられる．田中・辻(16)は稲熱病菌に関して galactose は kinase の作用により一旦 glucose に転位された後に利用されるが，xylose は glucose と同一酵素系によつて利用されることを報告している．本実験結果に於いては glucose と galactose は略同様に利用されている故に，kinase の作用による galactose から glucose えの転移が非常に容易に行われるか，或は galactose が glucose と同じ段階で代謝過程に入るかの何れかが考えられる．又 xylose は glucose と多少の相違はあるが，同一酵素系によつて分解されるのではなかろうかと思われる．以上の様に考えるならば，本菌による代謝過程が必ずしも前述した様な第一段階で糖類が enol 型に変化する様な直接酸化的代謝過程と一致するとは限らない． 特に arabinose は他の糖類と構造上かなりの相違がある故に，恐らく一部異つた代謝過程によつて分解，利用されるものと考えられる．

菌類にとつて炭素源の価値は，その炭素源が構成的代謝のため，生活維持の力源的代謝のため，或は構成仕事の力源的代謝のためによく用いられるか否かによつて決定される．しかしこれらは培養条件，菌類の特性，炭素源の化学的性質によつて左右せられる(12). しかしこれらを個々に論ずるのではなく，全体の影響としての菌類の生育の良否によつて決するのである．菌類の生育度を調べる場合に，その標準として我々は経済率(11)や経済効果(5)等を考えるのである．本菌による種々の炭素源の経済率に関しては前述の如く，単糖類と複糖類との間には明かに有意差があり，後者の方が大である．本菌も複糖類を加水分解した後に利用するとすれば，加水分解産物としての単糖類が然らざるものよりも適当な炭素源となりうることが判る．菌体生産の非常に劣つている arabinose が単糖類中で最も経済率が大である点に関しては，本実験の培養条件が arabinose の利用に好適であつたか，或は arabinose のみが他の糖類とその代謝過程が一部異る為であろう． 経済率は温度，培養液の濃度，pH 等によつて影響せられるところが大であるから，一回の培養試験で求められた経済率のみで炭素源の価値を決定するのは不適当である．炭素源として良好なものは呼吸材料としても適当でなければならないから，培養条件を変えて追試すると同時に呼吸材料としての適否を調べ，更に本菌の代謝過程を知らねばならない．

菌の生育に及ぼす炭素源濃度の影響に就いては栃内・中野(18)の稲熱病菌に関する報告があり，glucose の濃度が 0.05〜0.7 mol の範囲では濃度の低い程菌体の生産が大であるが，本実験は 1〜5％ の範囲では濃度が大なる程大である．これらの相違は菌の生育に及ぼす炭素源の濃度の影響が菌の種類によつて異る為であろう．培養温度の影響に就いては西門(9)は本菌を用いて 27〜30℃ で菌叢直径が最大であることを報じてあるが，本実験結果とよく一致している．しかし glucose 濃度が 1％ の場合，培養 15 日後の菌体生産は 28℃ の適温よりも低い温度で高い値を示しているが，この事は赤井(1)が既に報じた如く，glucose 含量が少い場合，15日間の培養では既に最高発育期を過ぎ，glucose が全部消費せられて，自己消化を始める為によるものと解せられる．

以上述べた結果から，菌類の栄養としての炭素源の価値は，経済効果のみで表わすのは不充分であり，種々の炭素源が利用される時の炭素源の消費，O_2 の吸収，CO_2 の放出，生産される中間産物の確認等から構成的物質代謝と力源的物質代謝の割合を直接調べ検討する必要がある．若し本菌に於ても pyruvate を経て TCA-cycle に入ると仮定するならば，TCA-cycle 中に含まれる種々の有機酸に関しても研究を拡げる必要がある．更に進んで本菌の行う代謝過程を調べ，種々の炭素源が如何なる過程を経て本菌個有の過程に入るかを知るべきである．炭素源に関する問題を取上げる限り，これらの問題は今後研究されるべき最も重要なものであらう．

5. 摘 要

1. 稲胡麻葉枯病菌 (*Cochliobolus miyabeanus*) の生育と培養基中の種々の炭素源との関係を調べ，更に各炭素源の経済率をも求めた．

2. Czapek 寒天培養基上での菌叢の発育は xylose, glucose, fructose, mannose, galactose, sucrose, maltose を含んだもので良好であるが，soluble starch, lactose ではやゝ劣る．glycerin では菌糸の発育，分生胞子の形成共にかなり不良であつた．

3. Czapek 液体培養基上では，菌体重の最大に達する日数や培養初期の生長の割合等から考えれば，maltose が最良の炭素源であり，fructose, sucrose, galactose, glucose, xylose 等がこれに次ぐ，一方 lactose はやゝ劣るが arabinose はこれらの炭素源中で最も不良である．

4. 炭素源の経済率に就いては，複糖類は単糖類よりも大であり，而もそれらの間には有意差が認められた．

5. glucose の濃度が 1～5％ の範囲内では濃度が高い程菌糸の発育良好であり，又培養温度は 28°C が最適である．

引 用 文 献

1. 赤井重恭：植物病害研究，4：64–70, 1951.
2. 朝井勇宣：化学の領域，6 (9)：6–18, 1952.
3. Blackmann, F. F.：Proc. Roy. Soc. Lon. sB. 103：491–523, 1928.
4. 藤井隆：科学，21 (10)：500–505, 1952.
5. Flieg, O.：Jahrb. wiss. Bot. 61：24–64, 1922.
6. Horr, W. H.：Plant Physiol. 11：81–99, 1936.
7. Lilly, V. G. and Barnett, H. L.：Physiology of the Fungi, 116–148, New York, Tronto, London, 1951.
8. Margolin, A. S.：The effect of various carbohydrates upon the growth of some fungi. thesis, West Virginia University, 1942.
9. 西門義一：日植病，1 (5)：20–30, 1923.
10. 大谷吉雄：日植病，17 (3,4)：119–120, 1953.
11. Pfeffer, W.：Jahrb. wiss. Bot. 28：205–268, 1885.
12. 坂村徹：植物生理学，上，309–415, 517–593, 東京，1950.
13. Steinberg, R. A.：Jour. Agr. Resarch. 59：749–763, 1939.
14. 滝本清透：微生物及び植物病理学実験法，東京，1950.
15. 田中正三・香月裕彦・香月文子：日植物，15 (3,4)：109–113, 1651.
16. 田中正三・辻二郎：日植病，16 (1)：31, 1952.
17. 田中正三・依田篤信：日化誌，73：8–10, 1952.
18. Tochinai, Y. and T. Nakano：Jour. Fac, Agr. Hokk. Imp. Univ. 44 (4)：183–229, 1940.
19. 富沢長次郎：日植病，17 (3,4)：113–118, 1953.

Résumé

1. In the present paper the investigation has been made on the relation between the different carbon sources supplied in culture media and the growth of *Cochliobolus miyabeanus*. The economic coefficient of the different carbon sources consumed by the present fungus was also calculated in order to decide the value of them as a nutrient.

2. The mycelial growth of the present fungus grown on Czapek's agar media containing xylose, glucose, fructose, mannose, galactose, sucrose, maltose as a carbon source respectively was very good, but not on soluble starch and lactose. On glycerin added media, the growth of the mycelium and the conidia formation were very poor.

3. Using Czapek's culture media, containing different carbon sources, the writer compared the maximum growth period of the mycelia and the growth rates in the earlier stage of the culture. From this result, maltose is the best among the carbon sources used and fructose, sucrose, galactose, glucose, xylose and lactose followed in descending order. Arabinose was the poorest among the carbon sources used.

4. The economic coefficient of disaccharides was larger than that of monosaccharides.

5. Within the limit of 1–5% of glucose added, the higher concentration it was, the better the fungus grew, and the optimum temperature for the mycelial growth lies at 28°C.

Sodium pentachlorophenate が土壌微生物に及ぼす影響

赤井重恭・奥　八郎

Shigeyasu Akai and Hatiro Oku: Influence of sodium pentachlorophenate upon the microflora in soil.

土壌に薬剤を施用した場合に，土壌中の病原菌は勿論，他のすべての微生物を殺すようなことがあれば，その土壌はもはや作物の生育に適しない dead soil となるかも知れない．Garber, Schaal & Fults[1] は pentachlorophenoxyacetic acid によつて *Streptomyces scabies* の防除を行う際に，この点に留意して実験を行つている．氏等によると，病原菌と非病原菌とを混じた培養基に pentachlorophenoxyacetic acid を加えた場合，薬剤が *Streptomyces scabies* の発育を阻止するため，それが分泌する抗生物質は減少する．結果として非病原性菌の発育は寧ろ増大するのを認めている．

筆者等は pentachlorophenol の 2, 3 誘導体を水稲の根部土壌に施用して，胡麻葉枯病に対する感受性の変化を観察したが，* 薬剤を土壌に施用した場合に，それが土壌微生物（細菌）に如何なる影響を及ぼすかを試験した．

実験圃場の土壌約 1g に水を 5 ml 加えて撹拌し，土壌浸出液を作り，次のような実験を行つた．

(1) 麦芽煎汁寒天培養基及びそれに 50 ppm の sodium pentachlorophenate を加えた培養基を加熱溶解して，45°C に保ちつつ，前記土壌浸出液を混じ，固化後 28°C に培養して，微生物（土壌細菌）の発育程度を比較した．

(2) 肉汁寒天培養基を前記と同様に処理して，36°C 下に培養して，微生物の発育を比較した．

上記の 2 培養基とも，同じような桿菌の発育をみたが，薬剤を加えない培養基 (control) では，多数の colony を生じたが，その中には methylene blue で染色し易いものと，染色し難いものとの 2 種を認めた．而して 50 ppm sodium pentachlorophenate を添加した media 上では大型の colony が少数発育したが，それ等は methylene blue で染色し易いものであつた．

このように sodium pentachlorophenate を添加した場合には，土壌細菌の colony 数は相当減少するが，すべてを殺すことはない．尚薬剤を添加した media 上で細菌 colony の大きいのは density effect によるものであろう．

下図は肉汁寒天培養基を 36°C 下で 3 日間保つて発育せしめた土壌細菌の colony である．（植病，第 78 号）

control　　　　　　　　0.05 % Na-P.C.P. 添加

Na-pentachlorophenate の土壌細菌に及ぼす影響

Effect of sodium-pentachlorophenate upon the microflora in soil.

(1) Phytopath. 41 : 991–996, 1951.　　* 赤井・奥：本誌 5 (4) : 159–163.

京大教授　赤井重恭監修

植物病害研究

第五集　第一號

Kyoto, Japan

1954

With the compliments of S. Akai

京都大学教授　赤 井 重 恭　監 修

植 物 病 害 研 究

第 五 集　第 二 号

Kyoto, Japan.

1955

植物病害研究　第5集 第2号

昭和 30 年 7 月 15 日　印刷
昭和 30 年 7 月 16 日　発行

京都大学農学部植物病理学研究室内
編集兼発行者　京都植物病学研究会
振替口座　京都三六九五番
代表者　赤井重恭

京都市下京区松原通高倉東入
印刷所　株式会社　芳文堂印刷所

With compliments of the author

FORSCHUNGEN
AUF DEM
GEBIET DER PFLANZENKRANKHEITEN

Herausgegeben von

Prof. Dr. Shigeyasu Akai
Universität zu Kyoto

Heft V, Nr. 3

Kyoto, Japan

1955

植物病害研究　第5集 第3号

昭和30年12月15日　印刷
昭和30年12月16日　発行

京都大学農学部植物病理学研究室内
編集兼発行者　京都植物病学研究会
振替口座　京都三六九五番
代表者　赤　井　重　恭
京都市下京区松原通高倉東入
印刷所　株式会社　芳文堂印刷所

With the compliments of S. Akai

FORSCHUNGEN
AUF DEM
GEBIET DER PFLANZENKRANKHEITEN

Herausgegeben von

Prof. Dr. Shigeyasu Akai

Universität zu Kyoto

Heft V, Nr. 4

Kyoto, Japan

1956